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Characterization of the pathological and biochemical markers that correlate to the clinical features of autism

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14. ABSTRACT The aim of this study was to characterize the type, topography and severity of developmental changes in the brain of people diagnosed with autism. To detect global pattern of brain pathology, the entire brain hemisphere was embedded in celloidin or polyethylene glycol and cut into hemispheric sections. Examination of CV stained serial sections revealed a broad spectrum of brain structure changes (defects of neurogenesis, migration, gyrification and dysplasia) affecting both gray and white matter in the brain and cerebellum of autistic subjects including: subependymal nodular dysplasia and thickening of subependymal layer; polymicrogyria; cerebellar hypoplasia and floculonodular dysplasia; broad spectrum of dysplastic changes in the neo- and archicortex, cornu Ammonis, dentate gyrus and amygdala; and heterotopias in the white matter in cerebellum, brain hemisphere and hippocampus. The study of 22 brains of subjects with idiopathic autism revealed changes in approximately 90% of cases and in all four examined brains of subjects with autism/Ch15Dupl. The type of detected changes indicates that all of these changes reflect fetal developmental alterations. Presence of these changes in autistic subjects, regardless of age, indicates that they are permanent alterations of brain structure and may contribute to the core clinical phenotype of autism.

15. SUBJECT TERMS Autism, Developmental Neuropathology, Subependymal nodular dysplasia, Heterotopia , Dysplasia

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Annual Report #2
October 21, 2010

Program Project Title: Characterization of the Pathological and Biochemical Markers that Correlate to the Clinical Features of Autism

Program Project PI: Jerzy Wegiel, Ph.D.; Co-PI: W. Ted Brown, M.D., Ph.D.

The overall aim of this multidisciplinary program project is to establish correlations between morphological and biochemical markers of autism and the clinical symptoms of the disorder.

Subproject 1

The neuropathological markers of abnormal brain development and aging in autism.

Subproject 1 P.I.: Thomas Wisniewski, M.D.

INTRODUCTION

This Program Project is focused on detection of:
(a) mechanisms leading to morphological changes and clinical autism phenotype,
(b) morphological and biochemical markers of autism,
(c) correlations between pathology and clinical manifestations of autism, and
(d) those pathological domains that might be a target for treatment.

Project 1 plays a dual role in the Program Project and its function is reflected in the technical and research aims:

Technical aims:
1. To preserve tissue from 56 brains, according to a standardized protocol for neuropathological studies (project # 1) and for morphometric studies (project# 2).
2. To implement clinical and neuropathological exclusion criteria to reduce the risk of results and conclusions distortion by comorbidity, postmortem tissue changes, and pathology associated with mechanisms leading to death.

Tissue preservation includes: brain hemisphere fixation, dehydration, embedding, cutting, staining/immunostaining. Two standardized protocols are applied: celloidin and polyethylene glycol (PEG) embedding protocols. The celloidin protocol provides 200-um-thick sections mainly for brain neuropathology and morphometry. The PEG protocol provides 50-um-thick sections for neuropathology and immunocytochemistry based morphometry (link between biochemistry and morphology/morphometry). The common denominator of both protocols is preservation of the entire brain hemisphere for a unique power (a) extended protocol of neuropathological evaluation and (b) complex

morphometric study of 17 brain regions selected to monitor potential link between structural developmental defects and three diagnostic domains of autism (social and communication deficits, and ritualistic behaviors) and intellectual deficits.

This process is monitored by a computerized system of brain tissue samples, sections and histological slides trafficking and storage. This Neuropathological Database is linked to the Project 2 Morphometric Database.

Research aims:

(a) To determine the type, topography and severity of developmental changes including defects of neurogenesis, migration and cytoarchitecture;

(b) To establish clinicopathological correlations and criteria for subclassification of the examined cohort according to clinical, neuropathological, morphometric and biochemical phenotypes. This aim will be executed by cooperation with the Principal Investigators of subproject 2 and 3.

BODY

Autism is characterized by a broad spectrum of clinical manifestations including:

(a) qualitative impairments in reciprocal social interactions,
(b) qualitative impairments in verbal and nonverbal communication,
(c) restricted repetitive and stereotyped patterns of behavior, interests and activities, and
(d) onset prior to the age of 3 years [1].

Studies of thousands of children has resulted in establishing the clinical diagnostic criteria of Pervasive Developmental Disorder including autism [1]. However, corresponding neuropathological diagnostic criteria do not exist. One of the reasons for the slow progress of neuropathological studies is limited tissue. Between 1980 and 2003, only 58 brains of individuals with autism were examined [15]. Due to diversity of research aims, of protocols of tissue preservation and of methods of sampling and examination, and the small number of brains examined in individual project (usually 2-5) the pattern of pathological changes emerging from these studies remains incomplete and inconsistent.

To overcome major weaknesses of previous studies, this Program Project is targeting on 68 brains (including 39 brains of subjects diagnosed with idiopathic autism and autism associated with chromosome 15 duplication, and 30 age-matched control brains). Therefore, this Program Project is expanding the world collection of brains examined postmortem with a new 39 brains of autistic subjects (67% increase).

Several reports published between 1980 and 1993 revealed that autism is associated with neuropathological changes [3, 7, 8, 9, 10, 11, 14, 17]. Current concept of autism related brain pathology integrates evidence of:

(a) abnormal acceleration of brain growth in early childhood [16],
(b) cortical minicolumn pathology [4, 5],
(c) curtailed neuronal development [3] and
(d) brain-structure specific delays of neuronal growth [19].

Moreover, abnormalities in brain cytoarchitecture [2,3], metabolic modifications with abnormal amyloid protein precursor (APP) processing [2,18], enhanced oxidative stress [6], and enhanced turnover of cell organelles with pigment accumulation and glial activation [12] were documented.

Hypothesis and aims. We combined the concept of "localizing" model of pathological changes within neuronal networks and brain structures [13] with a "developmental model" of defective neurogenesis, neuronal migration, and brain region-specific cytoarchitecture formation. Therefore, the aim of this study is both, to detect focal qualitative developmental defects (to identify structures and brain regions prone to developmental alterations in autism) and to sub-classify developmental defects as a signs of defective neurogenesis, migration, and maturation/networking.

Material and methods.

Tissue acquisition. The number of brains of subjects diagnosed with idiopathic autism, autism associated with Ch15 duplication, and control subjects, collected and preserved in the past two years has been expanded to 68.

Correction of tissue acquisition plan. The correction of the number of brains from the 56 proposed in our original Statement of Work to 68 is justified by the results of our neuropathological studies. This is the first morphological/morphometric study using rigorous clinical inclusion criteria and neuropathological exclusion criteria. We detected that about 31% of brains assigned to the project did not meet clinical inclusion criteria or neuropathological exclusion criteria. Neuropathology revealed that brain morphology is often distorted by comorbidity, mechanisms of death related pathology (ischemia/hypoxia- respiratory brain), or severe postmortem autolytic changes. Because these factors mask or distort some morphological markers of developmental defects (mainly quantitative changes such as cell number and volume), we made a decision to compensate losses caused by exclusions by adding more brains.

Thanks to the cooperation of the Autism Tissue Program that provided the clinical characteristics of patients and tissue donated by families of affected subjects, the structure and size of cohort was corrected consistently with the research design of the Program Project.

Therefore the Program Project exceeded in the second year the goals of the entire 3-year long project by 21%.

Consistently with the original research design and the Statement of Work, the study is developing in both directions:

(a) To increase the statistical power,
(b) To reduce bias associated with clinical diagnostic imperfections and postmortem studies.

We consider the applied research design as a model for future postmortem morphological, morphometric and biochemical studies that will reduce error and costs.

Progress of work and results.

Progress of work is consistent with the original Program Project and Project 1 aims and timetable.

The progress of collecting material, processing, embedding, cutting, staining, examination in an extended neuropathological protocol and results of neuropathological examination are summarized in 9 tables and illustrated by Figures 1-5.

PART I: IDIOPATHIC AUTISM. NEUROPATHOLOGICAL STUDY OF BRAINS PRESERVED IN THE CELLOIDIN PROTOCOL

Detailed review of results was presented in the first report. However, because the paper was published in 2010 [20] (see attachment Wegiel et al 2010), we summarize results in short summary below, and Table 1 and 2).

Aim: The aim of this study was to detect the patterns of focal qualitative developmental defects and to identify brain regions that are prone to developmental alterations in autism.

Material: Formalin-fixed brain hemispheres of 13 autistic (4–60 years of age) and 14 age-matched control subjects were embedded in celloidin and cut into 200-μm-thick coronal sections, which were stained with cresyl violet and used for neuropathological evaluation.

Defects of neurogenesis. Thickening of the subependymal cell layer in two brains and subependymal nodular dysplasia in one brain, are indicative of active neurogenesis in some autistic children.

Defects of migration. Subcortical, periventricular, hippocampal and cerebellar heterotopias detected in the brains of four autistic subjects (31%) reflect abnormal neuronal migration.

Defects of cytoarchitecture. Multifocal cerebral dysplasia resulted in local distortion of the cytoarchitecture of the neocortex in four brains (31%), of the entorhinal cortex in two brains (15%), of the cornu Ammonis in four brains and of the dentate gyrus in two brains. Cerebellar floculonodular dysplasia detected in six subjects (46%) and hypoplasia in one case indicate local failure of cerebellar development in 54% of autistic subjects.

Conclusion. Detection of floculonodular dysplasia in only one control subject and of a broad spectrum of focal qualitative neuropathological developmental changes in 12 of 13 examined brains of autistic subjects (92%) reflects multiregional dysregulation of neurogenesis, neuronal migration and maturation in autism, which may contribute to the heterogeneity of the clinical phenotype.

The outcome of this study. The results of application of clinical inclusion criteria and neuropathological exclusion criteria were the reduction of the size of cohort approved for morphometric studies from 20 to 13 autistic subjects and from 18 to 14 control subjects. Application of extended neuropathological study results both in a significant expansion of the list of detected developmental defects and identification of brain samples with severe death related changes and postmortem changes that may distort results of morphometric studies (Project 2).

PART II: IDIOPATHIC AUTISM (PEG PROTOCOL).

Examination of celloidin embedded material closes the first phase of the project. In the second phase brain tissue preserved in PEG protocol is examined **(Tables 3 to 6; Figures 1-5)**. Consistently with the plan, this subproject will be continued in 2011.

Aim 1: The aim of PEG protocol application is to preserve material for neuropathology-immunocytochemistry-morphometry based approach to characterize mechanisms leading to clinical deficits observed in autistic subjects.

200-um-thick sections from the celloidin protocol have an advantage of good brain anatomy and cytoarchitecture preservation for CV-based morphometry. The disadvantage is the sections' thickness that prohibit application of immunocytochemistry. The aim of immunostaining of free-floating 50-μm-thick PEG sections is to create a bridge between neuropathology, morphometry, biochemistry and genetics.

Aim 2a: To identify brain structures affected by developmental changes including: defects of neurogenesis (subependymal nodular dysplasia), migration (heterotopias) and abnormal cytoarchitecture (dysplastic changes) (Project 1), and to detect quantitative alterations in immunocytochemical and morphometric studies (Project 2).

Aim 2b: To identify brain samples with pathology related to mechanism of death and postmortem autolytic changes (Project 1) potentially affecting morphometric studies. Application of rigorous exclusion criteria will reduce the bias of morphometric studies (Project 2).

Material: Formalin-fixed brain hemispheres of 15 autistic (3–49 years of age) and 6 control subjects were embedded in PEG and cut into 50-μm-thick coronal sections (Tables 3, figure 1). 35,383 sections were preserved in the autistic group and 2,572 were CV stained. 12,493 sections were cut from the control brains and 901 were stained with CV. CV stained sections were used for the expanded neuropathological evaluation (Table 4).

Results

Defects of neurogenesis. Severe subependymal nodular dysplasia in the brain of a 32-year-old subject diagnosed with autism is a strong indicator of active neurogenesis in the brain of adult subjects with autism (Table 5, Figure 5).

Defects of migration. Subcortical, periventricular, hippocampal and cerebellar heterotopias detected in the brains of five autistic subjects reflect abnormal neuronal migration in 55% of examined autistic subjects. Presence of multiple heterotopias in the cerebellar white matter of three subjects indicates that 33% of autistic subjects are affected by cerebellar defects of migration (Table 5, Figure 5).

Defects of cytoarchitecture. Focal polymicrogyria affecting frontal and temporal lobe of an 8-year old male indicates that brains of autistic subjects are also prone to major defects of cerebral cortex gyrification (Table 5, Figure 4). Multifocal cerebral dysplasia resulted in local distortion of the cytoarchitecture of the neocortex in four brains (44%; Figure 3), of the entorhinal cortex in one brain (11%; Figure 2), and of the cornu Ammonis in two brains (22%; Fig. 2). Cerebellar floculonodular dysplasia detected in six

subjects indicate failure of development of lobe X in 66% of autistic subjects (Table 5, Figure 4).

Changes related to mechanism of death. Two brain samples had signs of severe generalized ischemic brain damage (neuronal necrosis, glial proliferation and activation, and neovascularization) related to survival on intensive support after drowning (3 and 11 years old autistic subjects; Table 6). These two brains have a full value in neuropathological evaluation but are of limited value in morphometric and biochemical studies. Other death- or other disease related changes affect brain structure locally and may limit application of tissue for some types of research.

Progress of lab work. Another six brains of autistic and control subjects are in different stages of processing, embedding, cutting, staining and evaluation (Table 5).

Conclusions.

1. Detection of a broad spectrum of focal qualitative neuropathological developmental changes in 8 of 9 examined autistic subjects in PEG protocol and 12 of 13 examined brains of autistic subjects in celloidin protocol reveals almost the same percentage of autistic people with developmental changes (89 and 92%, respectively) as in these two subprojects.
2. Detection of the next case with subependymal nodular dysplasia indicates that severe defects of neurogenesis are present not only in autistic children (5 years) but also in autistic adults (32 years of age).
3. Detection of polymicrogyria expands the list of fetal developmental defects and indicates that mechanisms leading to autism may alter neocortical gyrification.

PART III. AUTISM ASSOCIATED WITH CHROMOSOME 15 DUPLICATION.

In contrast to the first two subprojects, the third major component of this project is concentrated on pattern of developmental defects in the brain of subjects diagnosed with autism but with a defined genetic etiology. Thanks to parents of children diagnosed with autism/chromosome 15 duplication (Ch15Dupl) (IsoDicentric 15 Exchange Advocacy Support; IDEAS) and support of the Autism Tissue Program of Autism Speaks, all tissue donated for postmortem study has been assigned to this project.

Aim: To determine whether the type, topography and severity of developmental neuropathological changes in autistic subjects without genetic background and those with Ch15Dupl is similar.

Hypotheses:

(a) Similar pattern of neuropathological changes may indicate that in autism, regardless of genetic background, the major signs of structural/functional defects are comparable

(b) Detection of significantly different pattern of neuropathological changes may indicate that clinical phenotype of autism might be the product of different etiologies, mechanisms, and structural alterations.

Material. The aim of this subproject requires merger of subprojects 2 and 3. Results of the study of:

(a) 9 brains of subjects from 9 to 39 years of age diagnosed with autism/Ch15Dupl (Table 7) will be compared with results of the study of
(b) 15 subjects from 3 to 49 years of age diagnosed with idiopathic autism, and
(c) 6 control subjects from 8 to 32 years of age (Table 4).

Currently these three groups are not represented proportionally. Moreover, we expect that the number of cases diagnosed with autism/Ch15dupl might be increased by 1-3 during the third year of the project. To correct disproportions between the numbers of subjects in these three groups, we plan to increase the size of the control cohort by 6 cases.

The common denominator of all three groups is the same standard of tissue preservation and evaluation. Formalin-fixed brain hemispheres were embedded in PEG and cut into 50-μm-thick coronal sections. Brains of subjects diagnosed with autism/Ch15Dupl were cut into 25,515 serial sections and 1,519 were stained with CV (Table 8). CV stained sections were used for neuropathological studies.

Results.

Developmental changes. The project is currently in progress, but partial results already indicate some differences between the pattern of developmental neuropathological changes in subjects with idiopathic autism and autism/Ch15Dupl (Table 9).

The most striking are signs of cerebellar hypoplasia and heterotopias detected in all four examined subjects with autism/Ch15Dupl. Moreover, nodular or floculonodular dysplasia was detected in three subjects and dysplasia in vermis in one case.

Developmental defects in brain hemisphere are less consistent. A broad spectrum of type and topography of changes was detected in a 11-year old subject with focal dysplasia in the neocortex, amygdala and dentate gyrus, and heterotopia in the alveus. Focal neocortical dysplasia was detected in one subject and hippocamal dysplasia in one other subject.

Changes related to mechanism of death. Subject who died from choking developed hypoxic-ischemic encephalopathy with severe neuronal necrosis, gliosis and neovascularization (B-7041; 20-year old).

Changes secondary to seizures. Moreover in the 26 year of age subject with seizures Chaslin's gliosis with focal loss of cortical neurons and glial proliferation was found.

Conclusion. The pattern of developmental abnormalities observed in cerebellum associated with Ch15Dupl indicate that:

(a) the prevalence of cerebellar developmental defects is 100%
(b) the signs of cerebellar hypoplasia are present in 100% of these cases
(c) heterotopias in cerebellar white matter are also present in 100% of these subjects.

Examination of all cases with Ch15Dupl is necessary to confirm this preliminary conclusion based on current data.

KEY RESEARCH ACCOMPLISHMENTS

(a) Results provide a baseline for understanding the mechanisms involved in abnormal brain development in the brain of subjects with idiopathic autism and autism with Ch15 duplication.

(b) The summary of results of this neuropathological study will provide the map of structures with the highest probability for detection of autism-related pathology, and a list of the types of the most common developmental changes in autism.

(c) This report will be addressed to neuropathologists and medical examiners to modify the list of diagnostic samples. Inclusion of the cerebellar lobe X (flocculus/nodulus) will result in detection of focal dysplasia in approximately 50% of postmortem studies of brains of autistic subjects. Inclusion of samples from the wall of the lateral ventricle will detect evidence of abnormal neurogenesis in approximately 10% of children and adults with autism.

REPORTABLE OUTCOMES

1. The neurobiological and neuropathological background of this Program Project is summarized in our book chapter (See Appendices):

Wegiel J, Wisniewski T, Chauhan A, Chauhan V, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Wierzba-Bobrowicz T, Cohen IL, London E, Brown WT. Type, topography and sequelae of neuropathological changes shaping clinical phenotype of autism. In: Autism: Oxidative Stress, Inflammation, and Immune Abnormalities. Ed.: Abha Chauhan, Ved Chauhan and W. Ted Brown. Taylor & Francis/CRC Press, Boca Raton, FL, 2010, pp. 1-34.

2. Results of neuropathological evaluation of brains of 13 autistic and 14 control subjects are summarized in our paper published in *Acta Neuropathologica* (see Appendices):

Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba Bobrowicz T, de Leon M, Saint Louis LA, Cohen IL, London E, Brown WT, Wisniewski T. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathologica, 2010, 119,755-770

3. Oral presentation. 9th Annual International Meeting for Autism Research, Philadelphia, PA; 2010. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Cohen IL, London E, Brown WT, Wisniewski T. Defects of neurogenesis, neuronal migration, and dysplastic changes in brains of autistic subjects.

CONCLUSIONS

1. The study revealed a broad spectrum of brain structure changes (defects of neurogenesis, migration, gyrification and dysplasia) affecting both gray and white matter in the brain and cerebellum of autistic subjects including:
    - Subependymal nodular dysplasia,
    - Thickening of subependymal layer,
    - Polymicrogyria,
    - Broad spectrum of cerebellar hypoplasia,
    - Floculonodular dysplasia,
    - Broad spectrum of neocortical and archicortex dysplasia,
    - Cornu Ammonis, dentate gyrus and amygdala dysplasia,
    - Heterotopias in the white matter in cerebellum, brain hemisphere and hippocampus.
2. Application of the extended neuropathological protocol shows that changes are present in approximately 90% of the subjects with idiopathic autism.
3. Application of same standards of neuropathological evaluation revealed developmental alterations in all subjects with autism/Ch15Dupl. The striking feature is the presence of hypoplasia and heterotopias in the cerebellum of all affected subjects, suggesting that the cerebellum is the most prone structure to Ch15Dupl-associated developmental alterations.
4. The type of detected changes indicates that all of these changes reflect fetal developmental alterations.
5. Presence of these changes in autistic subjects, regardless of age, indicates that they are permanent alterations of brain structure and may contribute to the core clinical phenotype of autism.

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APPENDICES

Appendix 1

Wegiel J, Wisniewski T, Chauhan A, Chauhan V, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Wierzba Bobrowicz T, Cohen IL, London E, Brown WT. Type, topography and sequelae of neuropathological changes shaping clinical phenotype of autism. Chapter in: Autism: Oxidative Stress, Inflammation, and Immune Abnormalities. Ed.: Abha Chauhan, Ved Chauhan and W. Ted Brown. Taylor & Francis/CRC Press, Boca Raton, FL. 2010 pp. 1-34.

Appendix 2

Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba Bobrowicz T, de Leon M, Saint Louis LA, Cohen IL, London E, Brown WT, Wisniewski T. The neuropathology of autism: defects of neurogenesis, and neuronal migration, and dysplastic changes. Acta Neuropathologica 2010,119,755-770

SUPPORTING DATA:

Table 1. Material processed using celloidin protocol (for Neuropathological evaluation in Project 1 and Morphometric evaluation in Project 2)

#	Group	Brain Bank #	Sex	Age	Cause of death	PMI (h)	H	Brain weight (g)
1	A	IBR425-02	M	4	Drowning	30	R	1,280
2	A	UMB-1627	F	5	Trauma, multiple injuries	13.2	R	1,390
3	A	B-6403	M	7	Drowning	25	R	1,610
4	A	B-5666	M	8	Rhabdomyosarcoma	22.2	R	1,570
5	A	B-5342	F	11	Drowning, seizure related	12.9	L	1,460
6	A	B-5535	M	13	Seizure related	8	L	1,470
7	A	B-6115	F	17	Cardiac arrest related to cardiomyopathy	25	L	1,580
8	A	UMB-1638	F	21	Seizure related respiratory failure	50	R	1,108
9	A	B-6337	M	22	Seizure related	25	R	1,375
10	A	IBR93-01	M	23	Status epilepticus related to respiratory failure	14	R	1,610
11	A	B-6212	M	36	Cardiac arrest	24	R	1,480
12	A	B-6276	M	56	Cardiac arrest	3.35	R	1,570
13	A	B-7090	M	60	Pancreatic cancer	26.5	R	1,210
1	C	B-6736	F	4	Acute bronchopneumonia	17	R	1,530
2	C	UMB-1499	F	4	Lymphocytic myocarditis	21	R	1,222
3	C	UMB-4898	M	7	Drowning	12	R	1,240
4	C	UMB-1708	F	8	Trauma, multiple injuries	20	R	1,222
5	C	BTB-3638	M	14	Electrocution	20	R	1,464
6	C	UMB-1843	F	15	Trauma, multiple injuries	9	R	1,250
7	C	UMB-1846	F	20	Trauma, multiple injuries	9	R	1,340
8	C	UMB-1646	M	23	Ruptured spleen	6	R	1,520
9	C	UMB-4543	M	29	Traumatic multiple injuries	13	R	1,514
10	C	UMB-1576	M	32	Trauma, compressional asphyxia	24	R	1,364
11	C	BTB-3899	M	48	Atherosclerotic heart disease	24	L	1,412
12	C	IBR252-02	M	51	Myocardial infarct	18	L	1,450
13	C	BTB-3983	M	52	Heart atherosclerosis	13	R	1,430
14	C	B-6874	M	64	Cardiac arrest	28	R	1,250

PMI – Postmortem Interval,
H – hemisphere
R–right; L– left

Table 2. Neuropathologiy of developmental abnormalities in the brains of the autistic subjects (celloidin protocol).

Brain Bank #	Age	Type and topography of developmental abnormalities
IBR425-02	4	No changes
UMB-1627	5	Focal neuronal heterotopia in white matter of the anterior cingulate gyrus.
B-6403	7	Subependymal nodular dysplasia in the wall of the occipital horn of the lateral ventricle. Two periventricular nodular heterotopias (2 and 4 mm in diameter) near the frontal horn of the lateral ventricle. Tuber-like expansion of the tail of caudate nucleus into the lumen of the lateral ventricle. Flocculonodular dysplasia affecting almost entire lobe.
B-5666	8	Cortical dysplasia in the middle and inferior temporal gyri with focal dyslamination, with clustering of dystrophic neurons and local neuronal deficit by up to 80%, Several focal dysplastic changes within CA with irregular loose neuronal aggregates or multilayer neuronal formations. Very significant neuronal dysplasia with change of size, shape and spatial orientation of all neurons within affected areas. Flocculonodular dysplasia affecting almost entire lobe.
B-5342	11	Focal cortical dysplasia. Dysplasia of the granule layer of the dentate gyrus. Subcortical heterotopia in the inferior frontal gyrus. Heterotopia in vermis and in cerebellar white matter.
B-5535	13	Thickening of the subependymal germinal zone. Focal dysplasia within CA1 pyramidal layer with neuronal deficit, and loss of pyramidal neurons morphology and spatial orientation. Multifocal dysplasia of the dentate gyrus with distortion of the shape of granule and molecular cell layers (loops, circles and other forms of dysfiguration). Focal dysplasia within vermis.
B-6115	17	Flocculonodular dysplasia affecting the majority of lobe volume. Angioma within occipital cortex.
UMB-1638	21	Focal dysplasia within CA1 with diffuse neuronal deficit but without glial activation.
B-6337	22	Minor focal flocculonodular dysplasia.
IBR93-01	23	Focal dysplasia within islands in the entorhinal cortex. Pineal gland cysts.
B-6212	36	Several areas of focal cortical dysplasia within frontal cortex (2.4 mm long) and insula (more than 2.4 mm long) with local loss of vertical and horizontal organization. Merger of ventral portion of the claustrum with insula. Flocculonodular dysplasia affecting the majority of lobe volume.
B-6276	56	Focal dysplasia within CA1 sector with clustering of dysplastic neurons or focal neuronal deficit. Focal heterotopia within stratum oriens. Flocculonodular dysplasia affecting approximately 70% of the lobe.
B-7090	60	Three focal dysplasias in the frontal cortex. Dysplasia of layers 1-3 in the entorhinal cortex with missing numerous islands of the stellate neurons. Severe hypoplasia of cerebellar lobes 1-4 with reduction of volume of the molecular and granule cell layers by half and deficit of Purkinje cells. Reduced convolutions within almost half of the dentate nucleus.

Excluded cases. 20 brains of autistic and 18 brains of control subjects were assigned to the celloidin protocol. Seven brains of autistic subjects and four brains of control subjects were excluded from this study due to clinical factors, death related alterations or postmortem changes.

Table 3. Brain samples of autistic and control subjects assigned to PEG protocol.

#	Group	Brain Bank #	Sex	Age	Cause of death	PMI (h)	H	Brain weight (g)
1	A	B-6399	M	3	Hypoxic ischemic brain injury related to drowning	4	R	1328
2	A	B-7002	F	5	Drowning	33	R	1360
3	A	HSB4640	M	8	Presumed asthma related	13	R	1740
4	A	B-6349	M	9	Cardiac arrest	4	R	1690
5	A	B5807	M	10	Seizure, cerebral edema	74.5	L	1580
6	A	CAL105	M	11	Hypoxic brain injury related to drowning		R	RH: 800g
7	A	B-5891	M	15	Aspiration pneumonia	2.5	L	1390
8	A	B7079	M	15	Hanging	23.2	R	1370
9	A	B-6115	F	17	Cardiac arrest (Cardiomyopathy)	25	R	1580
10	A	B-6184	F	19	Related to seizure	6.7	L	2100
11	A	IBR93-01	M	23	Seizure (suspected)	14	L	1610
12	A	B-6994	M	28	Seizure (suspected)	43	R	1580
13	A	NP 06-54	M	32	Brain tumor (glioblastoma multiforme)		L	1260
14	A	B-6401	M	39	Cardiac arrest. Dissecting ascending aorta	13.9	R	1520
15	A	B-6469	F	49	Respiratory failure Metastatic brest carcinoma	16.3	R	1765
1	C	UMB-1706	F	8	Rejection of cardiac allograft	20	R	1340
2	C	PL M1-10	M	10	Carbon monoxide poisoning	-	R	RH: 560
3	C	UMB-1670	M	14	Hanging	5	R	1420
4	C	UMB4722	M	14	Multiple injuries	16	R	RH: 701
5	C	BTB3960	F	25	Not determined	26	L	1520
6	C	PL 291-00	M	32	Congestive heart failure. Aortic valve stenosis. Ventricular fibrillation.	14	R	1401

PMI – Postmortem Interval,
H – hemisphere
R–right; L– left

Table 4. Progress of brain tissue of autistic and control subjects processing, embedding in PEG, cutting, and staining with cresyl violet

#	Group	Brain Bank #	Sex	Age	ETOH dehydration (from – to)	Date of embedding in PEG	Number of sections cut	Number of CV slides
1	A	B-6399	M	3	11.06.07-11.27.07	11.30.07	3,408	200
2	A	B-7002	F	5	6.07.08- 7.03.08	07.09.08	4,140	207
3	A	HSB4640	M	8	10.02.07-10.26.07	10.26.07	4,892	288
4	A	B-6349	M	9	11.06.07-11.26.07	11.30.07	4,649	233
5	A	B5807	M	10	8.05.10- 8.12.10	08.11.10		
6	A	CAL105	M	11	4.12.06- 5.05.06	05.09.06	4,240	212
7	A	B-5891	M	15	8.05.10- 8.12.10	11.08.10		
8	A	B7079	M	15	8.05.10- 8.12.10	11.08.10		
9	A	B-6115	F	17	2.09.10- 3.05.10	04.05.10		
10	A	B-6184	F	18	6.17.08- 7.03.08	07.09.08		
11	A	IBR93-01	M	23	4.04.01- 4.20.01	04.25.01	8,497	629
12	A	B-6994	M	29	11.14.07-11.30.07	12.06.07	4,391	220
13	A	NP 06-54	M	32	7.04.09- 7.24.09	07.27.09	1166	583
14	A	B-6401	M	39	2.09.10- 3.05.10	03.08.10		
15	A	B-6469	F	49	10.14.09-11.06.09	11.09.09	5,480	274
1	C	UMB-1706	F	8	10.02.07-10-20-07	10.26.07	3,791	190
2	C	PL M1-10	M	10	3.16.10- 4.09.10	04.12.10		
3	C	UMB-1670	M	14	10.02.07-10-20-07	10.26.07	4,752	238
4	C	UMB4722	M	14				
5	C	BTB3960	F	25	6.15.07- 7.12.07	07.16.07	3,340	168
6	C	PL 291-00	M	32	7.26.00- 8.12.00	08.16.00	610	305

PMI – Postmortem Interval,
H – hemisphere
R–right; L– left

Table 5. Developmental abnormalities detected in the brains of the autistic subjects (PEG protocol)

Brain Bank #	Age	Type and topography of developmental abnormalities
B-6399	3	Focal cortical dysplasia in the frontal cortex. Floculonodular (lobe X) dysplasia in the cerebellum. Heterotopias in the cerebellar white matter.
B-7002	5	Multifocal dysplasia in the archicortex (entorhinal cortex) and neocortex (insula and temporal lobe).
HSB4640	8	Focal polymicrogyria in the frontal lobe. Focal dysplasia in frontal and temporal cortex. Heterotopia in the alveus (hippocampus). Floculonodular (lobe X) dysplasia in the cerebellum.
B-6349	9	Cerebellar developmental defects with cortical dysplasia in the nodulus and multiple heterotopias in the cerebellar white matter.
CAL105	11	None
IBR93-01	23	Right hemisphere: heterotopia in the hippocampus. LH. Cortical dysplasia in the left nodulus of the cerebellum. Numerous cysts within pineal gland.
B-6994	28	Focal dysplasia in the temporal cortex. Cortical dysplasia in the nodulus. Numerous heterotopias in the cerebellar white matter.
NP 06-54	32	Nodular subependymal dysplasia in the frontal and occipital horn of lateral ventricle.
B-6469	49	Cortical dysplasia in the cerebellar nodulus.
Six brains are in different stages of processing. After cutting, staining and immunostaining they will be examined by neuropathologists (Project 1) and morphometrists (Project 2).		
B-6115	17	Right hemisphere embedded in PEG 4.05.10
B-6401	39	Right hemisphere embedded in PEG 4.05.10
B-6184	19	Left hemisphere embedded in PEG 7.29.08
B-5891	15	Tissue currently in processing. Date of embedding in PEG: 11.08.10
B5807	10	Tissue currently in processing. Date of embedding in PEG: 11.08.10
B7079	15	Tissue currently in processing. Date of embedding in PEG: 11.08.10

Table 6. Results of neuropathological evaluation of changes related to the cause mechanisms leading to death and affecting brain structure (Autistic subjects; PEG protocol).

Brain Bank #	Age	Type and topography of neuropathological changes related to the cause of death ar other types of neuropathological changes.
B-6399	3	Severe generalized ischemic brain damage with multiple foci of necrosis, glial reaction and neovascularization related to mechanism of death (cardiac arrest due to drowning, and several hours long resuscitation).
B-7002	5	Recent hypoxic changes in the CA1 sector related to mechanism of death (drowning).
HSB4640	8	Focal mild neuronal hypoxic changes in the archi- and neocortex related most likely to asthma.
B-6349	9	Mild brain edema due to cardiopulmonary arrest.
CAL105	11	Generalized ischemic brain damage with selective neuronal necrosis, neuronal ischemic changes without glial reaction caused by drowning, cardiac arrest and survival on support for 14 h.
IBR93-01	23	No changes
B-6994	28	No changes
NP 06-54	32	Brain tumor. Glioblastoma multiforme in right hemisphere temporo-occipito-parietal area.
B-6469	49	Multiple breast cancer micrometastases to the brain with multiple neocortical microinfarcts. Other: incipient age-associated neurofibrillary degeneration.

Table 7. Brain samples of subjects diagnosed with autism and chromosome 15 duplication assigned to PEG protocol.

#	Group	Brain Bank #	Sex	Age	Cause of death	PMI (h)	H	Brain weight (g)
1	A/15q	B-7359	M	9	Cardiac arrest. Seizure	13.63	R	1,130
2	A/15q	B-7741	M	10		17.7	R	1,070
3	A/15q	B-7014	M	11	Seizure related	10.5	R	1,540
4	A/15q	B-6973	F	15	Seizure suspected	24	L and R	1,120
5	A/15q	B-7619	F	15	Aspiration		L	
6	A/15q	B-7041	M	20	Choking on food	28.08	L	1,190
7	A/15q	B-7436	M	24	Sudden unexpected death (probably seizure related)	36.36	L	1,730
8	A/15q	B-6856	F	26	Asphyxia seizure related	28.67	R	1,310
9	A/15q	B-7723	F	39	Pneumonia	32.83	R	890

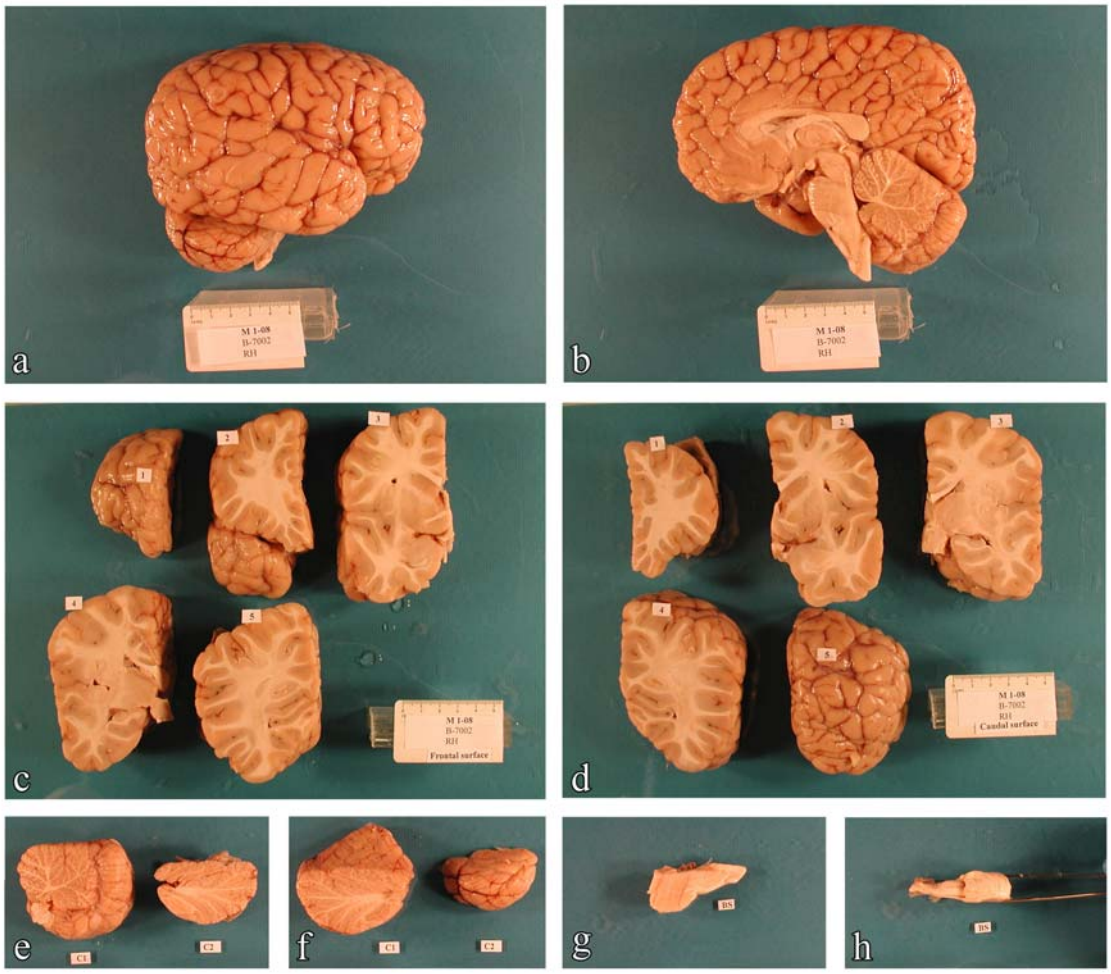
Table 8. Autism/Ch15 duplication. Progress of tissue acquisition, processing, embedding in PEG, cutting, and staining.

#	Group	Brain Bank #	Age	ETOH dehydration From - to	Date of embedding in PEG	Number of sections cut	Number of CV slides
1	A/15q	B-7359	9	3.26.10 – 4.12.10	5.10.10	2,990	180
2	A/15q	B-7741	10	8.03.10-8.26.10	9.23.10		
3	A/15q	B-7014	11	10.02.07-10.26.07	11.15.07	4,910	246
4	A/15q	B-6973	15	6.15.07-7.16.07	8.13.07	L:4,230 R:4,000	L: 423 R: 200
5	A/15q	B-7619	15	3.16.10-4.12.10	5.10.10		
6	A/15q	B-7041	20	11.14.07-12.06.07	12.26.07	4,505	226
7	A/15q	B-7436	24	3.16.10-4.12.10	5.10.10		
8	A/15q	B-6856	26	6.01.07- 6.27.07	7.17.07	4,880	244
9	A/15q	B-7723	39	8.03.10-8.26.10	9.23.10		

Table 9. Developmental abnormalities detected in the brains of subjects diagnosed with autism/Ch15Dupl.

Brain Bank #	Age	Type and topography of developmental abnormalities	
		Brain hemisphere	Cerebellar hemisphere
B-7014	11	Focal dysplasia with disorganization of neocortex, amygdala and the dentate gyrus cytoarchitecture. Heterotopia in hippocampal alveus.	Focal cerebellar hypoplasia. Heterotopias in the white matter. Nodular lobe dysplasia.
B-6973	15	Focal dysplasia with neocortical disorganization	Focal hypoplasia. Heterotopia in the white matter in left cerebellar hemisphere. Nodular lobe dysplasia in the right cerebellar hemisphere.
B-7041	20		Focal hypoplasia. Dysplasia in vermis. Subependymal heterotopia in the cerebellum.
B-6856	26	Focal dysplasia in the hippocampus.	Focal hypoplasia. Flocculonodular dysplasia. Heterotopia.

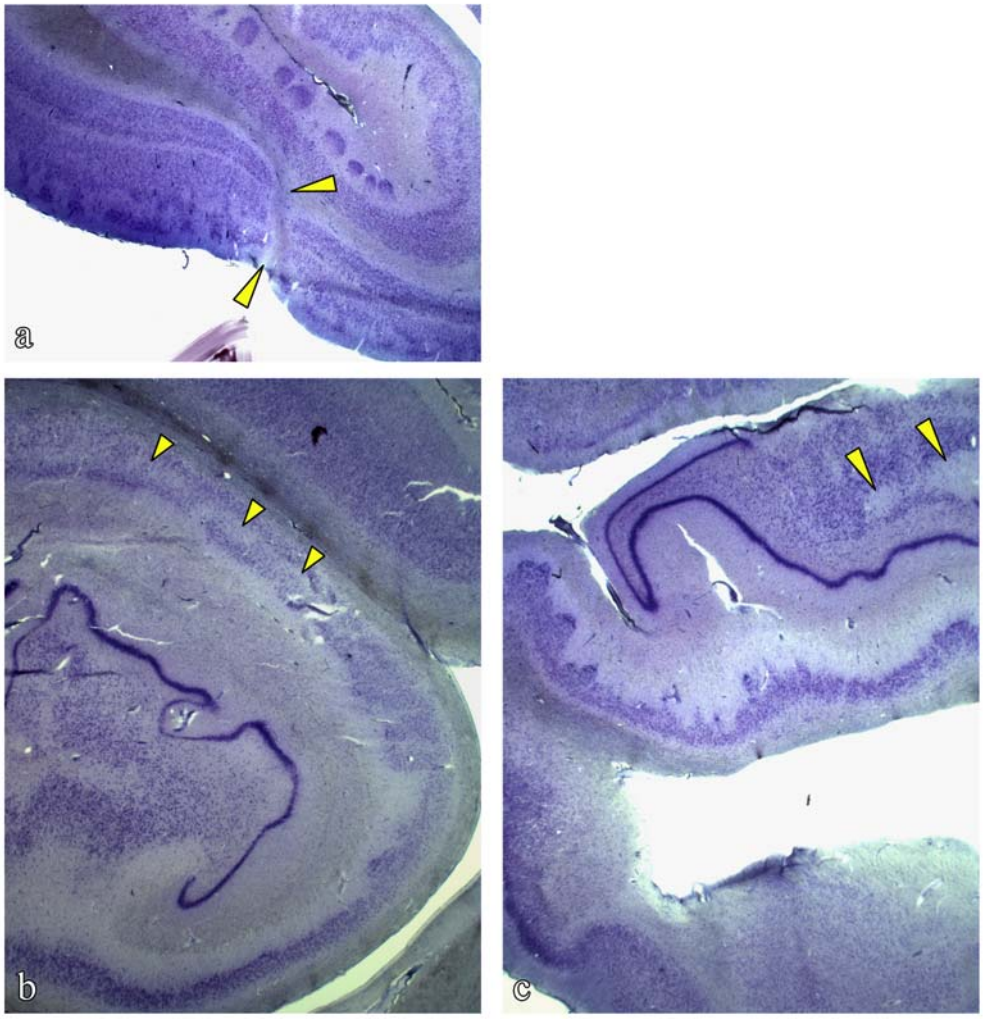
Figure 1



B-7002 Autism 5y F

To improve penetration of ethyl alcohol and PEG the brain hemisphere was dissected into five tissue blocks and cerebellum into two tissue blocks The brain was cut in frontal plane, the cerebellum in mid-saggital plane, and the brainstem in transverse plain. 50-um-thick serial sections were preserved for neuropathological and morphometric studies. Figure illustrates disproportions between temporal superior, middle and inferior temporal gyri (reduced size of the rostral portion of the temporal superior gyrus and disproportionally wide temporal middle gyrus).

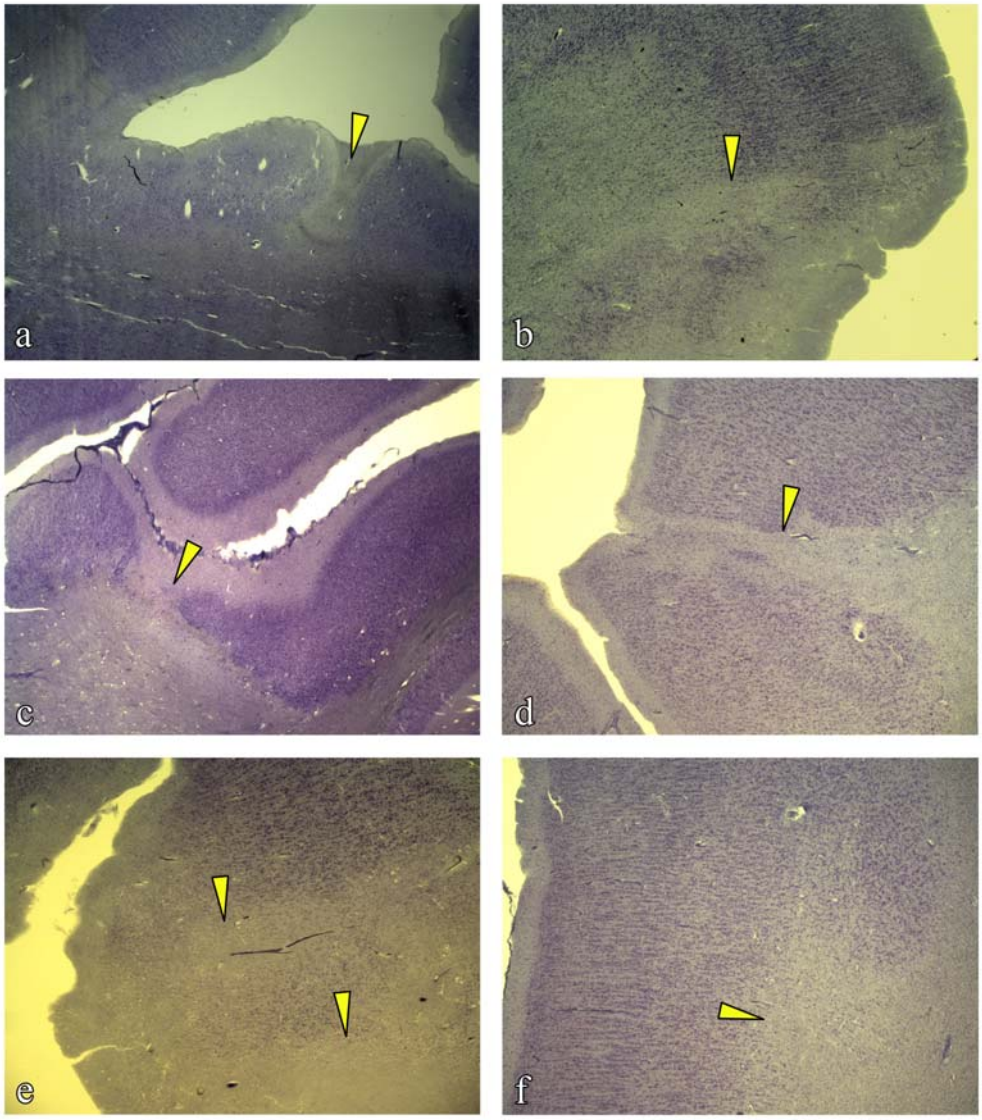
Figure 2



B7002 Autism 5y F

Five-year old autistic female. Loss of laminar continuity within the entorhinal cortex affecting all deep layers but without significant changes within island of stellate neurons (a, arrows). Focal hypocellularity dividing the pyramidal layer of the cornu Ammonis into the external and internal layer (b, arrows). Focal neuronal deficit within CA4 (c, arrows).

Figure 3

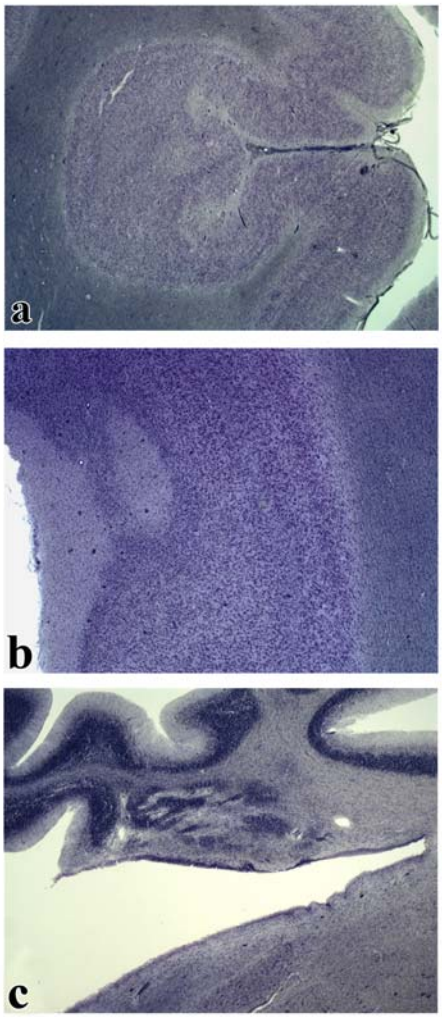


B7002 Autism 5y F

Five-year old autistic female. Dysplastic changes within the insula with (a) a gap in cortex continuity, (b) diffuse deficit of neurons, (c), segmental lack of cortical cytoarchitecture and thinning of cortex. Focal cortical dysplasia in the temporal superior gyrus with columnar (d) or diffuse (e) neuronal deficit. Focall hypocellularity in layers V and VI in the temporal superior gyrus (f).

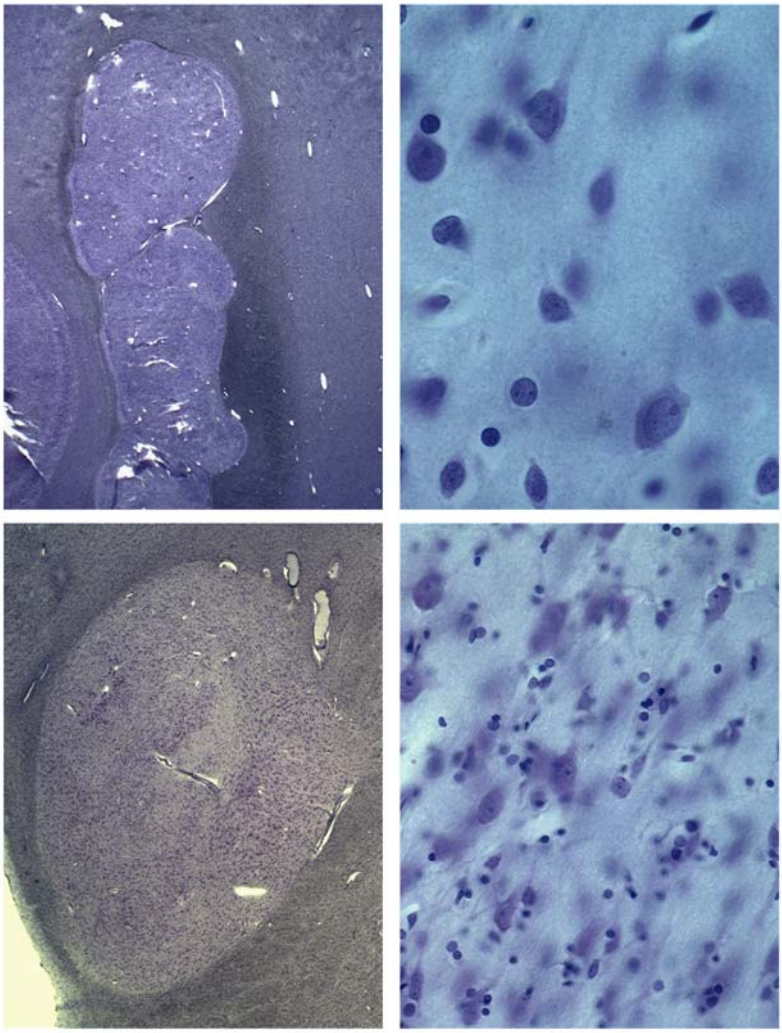
Eight-year old autistic subject. Abnormal folding of the cerebral cortex between the frontal gyrus and orbital gyrus with defects of lamination corresponding to focal polymicrogyria (a). Focal cortical dysplasia in the superior frontal gyrus (b). Flocculonodular dysplasia in the cerebellum of 8-year old autistic subject (c).

Figure 4



HSB4640 Autism 8y

Figure 5



NP06-54 Autism 32y M

Numerous nodules were found within the subependymal layer in the wall of the occipital horn of the lateral ventricle of 32-year old autistic subject (subependymal nodular dysplasia). Nodules contained dysplastic neurons with morphology of pyramidal, multipolar or small granule-like neurons (upper row). Periventricular heterotopia, 3-mm in diameter, composed of mature small and large neurons with random spatial orientation, was detected near the frontal horn of the lateral ventricle (bottom row).

1 Type, Topography, and Sequelae of Neuropathological Changes Shaping Clinical Phenotype of Autism

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1.1 INTRODUCTION

The aim of this chapter is to identify the type, topography, and sequelae of neuropathological changes that contribute to the clinical phenotype of autism. Results of recent magnetic resonance imaging (MRI) and postmortem neuropathological and stereological studies of autism brain suggest a dynamic model of sequential subdivision of age- and brain-specific structural and functional changes. Acceleration of brain growth in the first year of life and deceleration in the second and third years appear to play a pivotal role in the onset of clinical signs of autism (Courchesne et al., 2001, 2003; Courchesne and Pierce, 2005b; Dawson et al., 2007; Dementieva et al., 2005; Gillberg and de Souza, 2002; Redcay and Courchesne, 2005). The range of deviation from the normal trajectory of brain growth may be a factor determining the severity of the disease (Courchesne et al., 2003). Developmental heterochronicity (differential rates of growth of various brain regions compared to controls), resulting in selective overgrowth of some brain regions, appears to be a key factor determining topography and brain region–specific type of cytoarchitectonic changes (Carper and Courchesne, 2005; Carper et al., 2002; Courchesne et al., 2001; Hazlett et al., 2005; Sparks et al., 2002). Topographic developmental heterochronicity may result in impairment of both local and global connectivity, leading to local overconnectivity and impairment of long-distance connectivity (Baron-Cohen, 2004; Casanova et al., 2006; Courchesne and Pierce, 2005a). Stereological studies have revealed neuronal developmental heterochronicity in early childhood, resulting in selective developmental delay of the growth of neurons in some subcortical structures and the cerebellum during the most critical stage of development of social behaviors and communication skills (Wegiel et al., 2008). Distortions of brain and neuronal development are reflected in abnormal

cortical minicolumn organization (Casanova et al., 2002, 2006), local dysgenesis, and ectopias (Bauman and Kemper, 1985; Bauman et al., 1997; Kemper and Bauman, 1993, 1998). These complex developmental abnormalities appear to lay the foundation for secondary and tertiary metabolic, structural, and functional changes, including seizures and risk of sudden unexpected death; signs of oxidative stress, early and enhanced accumulation of products of cell organelle degradation with lipofuscin deposition; modified processing of β-amyloid precursor protein with accumulation of truncated amyloid beta; and other as of yet unidentified changes. Secondary pathologic changes appear to be indicators of the susceptibility of abnormally developing neurons to further modifications during cell maturation and aging. The pattern of morphological changes emerging from these multidisciplinary studies appears to represent a major trend. However, modifications of the course of disease and subpatterns of developmental changes result in a broad spectrum of morphological and clinical interindividual differences.

1.2 CLINICAL, ETIOLOGICAL, AND NEUROPATHOLOGICAL DIVERSITY IN AUTISM

Autism is the prototype of a pervasive developmental disorder (PDD) and is characterized by (a) qualitative impairments in reciprocal social interactions, (b) qualitative impairments in verbal and nonverbal communication, (c) restricted repetitive and stereotyped patterns of behavior, interests, and activities, and (d) onset prior to the age of 3 years. PDD also includes childhood disintegrative disorder, Asperger's disorder, Rett syndrome, and pervasive developmental disorder—not otherwise specified (PDD-NOS). The common features of all these disorders are qualitative deficits in social behavior and communication (American Psychiatric Association, 2000).

1.2.1 Clinic

In most cases (90%–95%), it is not presently possible to detect a known or specific etiology. These cases are referred to as idiopathic or nonsyndromic autism (Boddaert et al., 2009; Gillberg and Coleman, 1996). In 6% (Fombonne, 2003), 5% (Tuchman et al., 1991), or 10% (Rutter et al., 1994) of cases, autism was diagnosed in association with other disorders. About 30% of children with idiopathic autism have complex autism, defined by the presence of dysmorphic features, microcephaly and/or a structural brain malformation (Miles et al., 2005). About 70% of children with autism have essential autism, defined by the absence of physical abnormalities. For most children, the onset of autism is gradual. However, a multisite study revealed significant regression at ages of 18 to 33 months (regressive autism) in about 13.8% (Colorado) to 31.6% (Utah) of autistic subjects (Department of Health and Human Services, 2007). Moreover, the manifestations of autism vary greatly, depending on developmental level and chronological age of the affected individual. The majority of patients exhibit serious social and communicative impairments throughout life but some improve enough to be able to live relatively independently as adults. In 44.6% of children, autism is associated with cognitive impairment (defined as having intelligence quotient scores of <70; Department of Health and Human Services, 2007). Expressive language function in

individuals with autism may vary from mutism to verbal fluency (Rapin, 1996; Stone et al., 1997; Wetherby et al., 1998). Sensorimotor deficits also show significant interindividual differences, with more frequent and severe impairments of gross and fine motor function (motor stereotypes, hypotonia, limbic apraxia) in subjects with lower IQ (Rogers et al., 1996). Hand mannerisms and body rocking are reported in 37% to 95% of individuals with autism (Lord and Rutter, 1995; Rapin, 1996; Rogers et al., 1996), whereas preoccupation with sensory features of objects, abnormal responsiveness to environmental stimuli, or paradoxical responses to sensory stimuli are seen in 42% to 88% of people with autism (Kientz and Dunn, 1997). Epilepsy is a comorbid complication, occurring in up to 33% of individuals with autism (Tuchman and Rapin, 2002).

1.2.2 Etiology

The clinical diversity of autism reflects the etiologic heterogeneity of this disorder. Genetic factors; pre-, peri-, and postnatal pathological factors; and concurrent diseases may contribute to autism (Muhle et al., 2004; Newschaffer et al., 2002; Rutter et al., 1994). About 5% to 10% of cases are associated with several distinct genetic conditions including fragile X syndrome, tuberous sclerosis, phenylketonuria, Rett syndrome, and chromosomal anomalies such as Down syndrome (DS) (Folstein and Rosen-Scheidley, 2001; Fombonne, 2003; Smalley et al., 1988; Yonan et al., 2003). Autism spectrum disorders (ASDs) in people with DS have been described in several reports (Ghaziuddin et al., 1992; Howlin et al., 1995; Pracher and Clarke, 1996; Wakabayashi, 1979), and the prevalence of autism in boys with DS was estimated as at least 7% (Kent et al., 1999). The prevalence of autism in the fragile X syndrome is estimated as 15%–28% (Hagerman, 2002). Cytogenetic abnormalities (partial duplications, deletions, inversions) in the 15q11-q13 region account for 1% to 4% of autism cases (Cook, 1998; Gillberg, 1998). Several potential candidate genes have been identified in both autosomes and X chromosomes, including the tuberous sclerosis gene on chromosomes 9 and 16; serotonin transporter on chromosome 17; gamma-aminobutyric acid receptor-beta 3 on chromosome 15; neuroligins on the X chromosome (see Vorstman et al., 2006); and possibly *PTEN* on chromosome 10 (Butler et al., 2005). Modifications in the tryptophan hydroxylase gene may play a modest role in autism susceptibility (Coon et al., 2005).

1.2.3 Neuropathology

While knowledge of the clinical and genetic factors in autism is based on examination of thousands of patients, postmortem neuropathological studies are based on reports of a very small number of brains. A review by Palmen et al. (2004) revealed that between 1980 and 2003, only 58 brains of individuals with autism have been examined, and results of only a few neuropathological and stereological studies were published. Usually, neuropathological reports and morphometric reports were based on evaluation of one or several brains. Due to the broad age spectrum and the etiological and clinical diversity in autism, the pattern of neuropathological changes reported is incomplete and often inconsistent. As a result, the morphological markers and

neuropathological diagnostic criteria of autism have not yet been established (Lord et al., 2000; Pickett and London, 2005). In the past, the contribution of postmortem studies to the detection and characterization of neuropathological changes and mechanisms leading to structural and functional manifestations of autism was limited because of (a) the deficit of autism brains, resulting in a lack of statistical power, (b) the lack of efficient mechanisms for sharing the limited tissue resources, (c) the lack of complex studies of the dynamic of changes during the life span, (d) the infrequent application of unbiased morphometric methods to detect quantitative differences, and (e) the averaging of results from subjects with different clinical and morphological manifestations of autism. Heterogeneity within the autism spectrum is the major obstacle to autism research at all levels (Newschaffer et al., 2002), including neuropathological studies and attempts at detection of clinicopathological correlations. Recent evidence of genetic fractionation of social impairment, communication difficulties, and rigid and repetitive behaviors indicates that heterogeneity in ASD could be an unavoidable consequence of the contribution of nonoverlapping genes. If different features of autism are caused by different genes associated with different brain regions and related to different core cognitive impairments (Happe et al., 2006), it seems likely that many brain networks are involved in the pathology of autism. The diversity of neuropathological findings and the commonly reported inconsistencies in regional findings correspond to developmental impairments in many interacting brain networks and to expansion from "local" abnormalities to "nonlocal" effects of the emerging cognitive system. In spite of these limitations, "localizing" models are still the main approach to the identification of pathological changes as a component of the structural and functional abnormalities of the networks (Müller, 2007).

The possibility that autism is associated with neuropathological changes was explored in the first studies reported between 1980 and 1989 (Bauman and Kemper, 1985; Courchesne et al., 1987, 1988; Damasio et al., 1980; Gaffney et al., 1987; Hashimoto et al., 1989, 1993; Murakami et al., 1989; Ritvo et al., 1986). Expansion of these studies through examination of larger cohorts and application of stereology, functional and structural MRI, and biochemistry resulted in the identification of several major forms of pathology contributing to the clinical phenotype including abnormal acceleration of brain growth in early childhood (Redcay and Courchesne, 2005), delay of neuronal growth (Wegiel et al., 2008), changes in brain cytoarchitecture (Bailey et al., 1998; Bauman and Kemper, 1985; Casanova et al., 2002, 2006), metabolic modifications with abnormal amyloid precursor protein (APP) processing (Bailey et al., 2008; Brown et al., 2008; Sokol et al., 2006), enhanced oxidative stress (reviewed in Chauhan and Chauhan, 2006), and turnover of cell organelle with pigment accumulation and glial activation (Lopez-Hurtado and Prieto, 2008).

1.3 DEREGULATION OF BRAIN GROWTH IN EARLY CHILDHOOD

The major measures of age-related changes are head circumference, MRI-based volumetry of the brain and brain structures, and postmortem brain weight and volume of brain subdivisions. Between 1990 and 2000, several groups reported increased head circumference (Bailey et al., 1995; Bolton et al., 1995; Davidovitch et al., 1996;

Fidler et al., 2000; Fombonne et al., 1999; Lainhart et al., 1997; Miles et al., 2000; Steg and Rapoport, 1975; Stevenson et al., 1997), whereas MRI-based studies revealed increased brain volume (Piven et al., 1995, 1996). According to Fombonne et al. (1999), the prevalence of macrocephaly in autism is about 20%. In a report by Bailey et al. (1998), four of six subjects with autism 4 to 24 years of age had macrocephaly. Increased brain weight was reported in postmortem studies by Bailey et al. (1993) and Kemper and Bauman (1998). Increase in the volume was regional and not generalized, with the greatest enlargement in the occipital and parietal lobes (Filipek, 1996; Filipek et al., 1999; Piven et al., 1995, 1996). However, in several studies, an increase in brain size was not detected (Garber and Ritvo, 1992; Haznedar et al., 2000). Inconsistency in detection of abnormal head and brain size can be associated with interindividual differences, the age of examined individuals, and the methods applied. Courchesne et al. (2003) integrated their work and that of other researchers into the concept of four phases of modified brain growth, described below.

At birth, the head circumference of neonates later diagnosed with autism is normal or slightly less than that observed in normally developing children (Courchesne et al., 2003; Dawson et al., 2007; Dementieva et al., 2005; Dissanayake et al., 2006; Gillberg and de Souza, 2002; Hazlett et al., 2005; Lainhart et al., 1997; Mason-Brothers et al., 1990; Stevenson et al., 1997). Slight undergrowth is independent of body growth and may be a reflection of prenatal neural developmental defects corresponding to pathology detected in postmortem studies of the brains of autistic adults (Bailey et al., 1998; Casanova et al., 2002; Courchesne et al., 2003; Kemper and Bauman, 1998). In only 5% of neonates diagnosed later as autistic was the head circumference more than that in normally developing infants (Courchesne et al., 2003; Dementieva et al., 2005).

In the second phase, by 1 or 2 years of age, a rapid and large increase in head circumference distinguished children diagnosed later with autism from normally developing children (Courchesne et al., 2003; Dawson et al., 2007; Dementieva et al., 2005; Dissanayake et al., 2006; Hazlett et al., 2005). Ninety percent of 2- and 3-year-old children with autism had brain volumes larger than those of control children (Courchesne at al., 2001). According to Dawson et al. (2007), a period of exceptionally rapid head growth is limited to the first year of life, and head growth decelerates after 12 months of age. Acceleration of growth in head circumference appears to begin at about 4 months (Courchesne and Pierce, 2005a; Gillberg and de Souza, 2002; Redcay and Courchesne, 2005). Using meta-analysis based on evaluation of head circumference converted to brain volume, brain volume measured from MRI, and brain weight from postmortem studies, Redcay and Courchesne (2005) revealed that brain size increases from 13% smaller than in control subjects at birth to 10% larger than in control infants at 1 year, but only 2% greater by adolescence. The greater growth rate of head circumference in the first year, and its return to normal rates thereafter, is not accounted for by an overall growth in stature. Studies of behavioral development in infants later diagnosed with autism suggest that the period of acceleration of head growth precedes and overlaps with the onset of behavioral changes, and that the period of deceleration coincides with a period of behavioral decline or worsening of symptoms in the second year of life (Dawson et al., 2007). Coincidence of acceleration of brain growth rate with onset and

worsening of clinical symptoms may indicate that structural developmental changes critical for a lifelong phenotype occur in early infancy. Acceleration of brain growth in the first year and deceleration in the second year of life suggest that failure of the mechanism controlling brain growth in the first year of life plays an essential role in the onset of clinical features of autism. Identification of these mechanisms may lead to conceptualization of early preventive treatments.

In the third phase, of 2 to 4 years, the overall rate of brain growth slows but is still 10% more than in normally developing children (Carper et al., 2002; Courchesne et al., 2001; Hazlett et al., 2005; Sparks et al., 2002). In 4- to 5-year-old autistic children, MRI-based estimated brain volume is 1350 mL, whereas in normally developing children, a comparable volume (1360 mL) is reached about 8 years later. In postmortem studies, the brain weight of 3- to 5-year-old autistic males was 15% higher (1451 g) than in control males of this age (1259 g) (Redcay and Courchesne, 2005).

In the fourth phase, the volume of the brain decreases, and this trend extends from middle/late childhood through adulthood. Head (Aylward et al., 2002) or brain enlargement (Bailey et al., 1998; Hardan et al., 2001; Lainhart et al., 1997; Piven et al., 1995, 1996) has also been observed in studies of older populations of autistic individuals. However, by adolescence and adulthood, the average size of the brain is only 1% to 3% greater in autistic than in control cohorts (Redcay and Courchesne, 2005).

Moreover, the pattern of brain growth reflects the severity of clinical manifestation of autism (Courchesne et al., 2003). Among infants who have the more severe form of autism, 71% showed increases during their first year of more than 1.5 S.D., with 59% showing increases between 2.0 and 4.3 S.D. In children with a less severe form of autism, PDD-NOS, acceleration of brain growth is observed later, and the increase is less pronounced. Later onset and slower rate of progression of autism appear to be associated with a better outcome.

1.3.1 Developmental Heterochronicity

Developmental heterochronicity studies indicate that autism is a disorder involving a transient period of pathological acceleration of brain growth. Developmental heterochronicity, with different rates of growth for different brain regions/structures, appears to be the second major factor contributing to the clinical phenotype. MRI studies showed that overgrowth of the frontal and temporal lobes and amygdala, brain regions that are involved in cognitive, social, and emotional functions as well as language development, is synchronized with brain overgrowth in 2- to 4-year-old autistic children in contrast to a different rate of growth of the occipital cortex (Carper and Courchesne, 2005; Carper et al., 2002; Courchesne et al., 2001; Hazlett et al., 2005; Sparks et al., 2002). The reduced size of the body and posterior subregions of the corpus callosum noted in subjects with autism may indicate disproportions in brain subregions development (Piven et al., 1997b). The cellular and molecular basis for transient acceleration of brain growth and enhanced growth of some brain regions is not known, but Courchesne et al. (2003) proposed that the observed pattern is associated with an excessive number of neurons, enhanced rate of growth of size of neurons, and increased number of minicolumns as well as excessive and premature expansion of the dendritic tree.

1.3.2 Functional Consequences of Abnormal Brain Development

Using a computational model analogue of autism, Cohen (2007) has argued that an interaction between stochastic and above-average or "excessive" numbers of neural connection factors has implications for understanding the disorder. In particular, a relative excess of connections could lead to enhanced recognition of complex patterns in the environment. In Cohen's chapter, it was noted that if large and complex brains are in part familial (Courchesne et al., 2003; Fidler et al., 2000), and brain size is heritable (Pfefferbaum et al., 2000) and positively correlated with IQ (Pennington et al., 2000), then behavioral outcomes both within and across generations of family members could result in (a) individuals who may be unusually gifted in their ability to handle complex nonlinear problems such as mathematics or computer science, (b) individuals with autism, or (c) individuals with a combination of autism or autistic-like behavior and giftedness (many typical Asperger's cases). These trends are detected among relatives of subjects with autism (Folstein and Rutter, 1988).

The effect of an abnormal trajectory of brain development observed in autism are well-validated characteristics of the learning style of children with autism, including (a) greater attention to idiosyncratic than socially relevant stimuli, (b) stimulus overselectivity or a lack of drive for central coherence, (c) problems with acquiring fuzzy concepts, (d) development of savant skills, (e) problems with generalization of previously acquired skills, (f) rigidity and resistance to change, (g) social and communication deficits, and (h) difficulty in learning complex higher-order concepts (Cohen, 2007).

1.4 CORTICAL AND SUBCORTICAL NEUROPATHOLOGY

1.4.1 Cortical Dysgenesis, Lamination Defects, Migration Disturbances

The fundamental characteristics of the neuropathological changes described by Kemper and Bauman (1993, 1998), Bauman and Kemper (1985, 1996), and Bauman et al. (1997) suggest three major neuropathologies in the brain of people with autism: (a) curtailed development of neurons in the structures that are substrates for memory and emotions—the entorhinal cortex, hippocampus, subiculum, anterior cingulate gyrus and mamillary body; (b) a congenital decrease in the number of Purkinje cells in the cerebellum; and (c) age-related differences in cell size and number of neurons in the cerebellar nuclei and in the inferior olivary nucleus. Microdysgenesis is represented by increased neuronal density in the cortical layer, clustering of cortical neurons, disorganization of cortical layers, neuron cytomegaly, ectopic neurons, and nodular heterotopias. A detailed study of serial sections from the brain of a 29-year-old man with autism revealed reduced neuronal size and increased cell-packing density (Bauman and Kemper, 1985), both features of an immature brain (Friede, 1975). Cell-packing density was increased by 66% in the hypothalamus and mamillary body, and by 54% in the medial septal nucleus, with smaller nerve cells. The reduced size of neurons and the selective increase in cell-packing density were seen in central (40%), medial (28%), and cortical nuclei (35%). Atrophy of the neocerebellar cortex, with marked loss of Purkinje cells and, to a lesser extent, of granule cells, was present in gracile, tonsil, and inferior semilunar lobules. Changes were not detected in

the anterior lobe or the vermis. Reduced numbers of cells were noticed in fastiglial, globose, and emboliform nuclei, and cells were small and pale. The dentate nucleus was distorted. Retrograde neuronal loss in the inferior olive related to neuronal loss in cerebellar cortex was not found, but olivary neurons were small and pale. Brain cytoarchitecture abnormalities were not associated with gliosis. In a 21-year-old female with autism, Rodier et al. (1996) found that the brain was smaller than a control brain, and the length of facial nerve nucleus was less than 500 μm as compared to 2610 μm in the control subject.

1.4.2 Brain Structure–Specific Delay of Neuronal Growth

The reduced size of neurons and their nuclei in the cortex of autistic subjects reported by Casanova et al. (2006) could be an indicator of reduced or impaired functional connectivity between distant cortical regions (Casanova et al., 2006; Just et al., 2004; Koshino et al., 2005). Our ongoing studies of series of brains from age-matched autistic and control subjects (Wegiel et al., 2008) indicate that reduced size of neurons is a brain structure–specific marker. In 4- to 7-year-old autistic children, Purkinje cells were smaller by 38%. Neurons in the dentate nucleus were reduced by 26%; in the amygdala, by 24%; in the nucleus accumbens, by 41%; in caudate, by 20%; and in the putamen, by 27%. Neurons in the nucleus of the facial nerve and the nucleus olivaris did not show a significant difference from controls. The second significant feature of the pattern of neuronal size abnormalities is the partial or complete correction of the size of neurons (for example, in the nucleus accumbens) observed in late childhood or adulthood. This study indicates that the delay of growth of neurons is the most consistent pathology detected in the brains of examined people with autism. Pathology is brain structure–specific. Changes may range from no delay to severe developmental delay. The youngest examined children (4 to 7 years old) show the most severe deficit in the volume of the neuronal body and nucleus. Partial correction of cell volume is observed in late childhood and adulthood, which indicates that brain structure and function undergo modifications during the life span. The study of basal ganglia and cerebellum supports the hypothesis that clinical manifestations of autism are the result of regional neuronal maldevelopment.

One may assume that mechanisms regulating growth of the neuron in early childhood are the target of factors that are the cause of autism. The result of deregulation of these mechanisms could be (a) significantly delayed growth of neuronal body, nucleus, dendritic tree, spines, and reduced number of synapses and (b) functional deficits corresponding to these structural developmental delays. These abnormalities of very early childhood might be the major contributor to clinical deficits that are the basis for the clinical diagnosis of autism at the age of 3 years.

1.4.3 Minicolumnar Abnormalities in Autism

The next significant contribution to detection of neocortical developmental pathology is the result of studies of minicolumns by Casanova's group (Buxhoeveden and Casanova, 2002; Casanova et al., 2002, 2006). Malformations of cortical development are observed in heterogeneous disorders caused by abnormalities of cell

proliferation, apoptosis, cell migration, cortical organization, and axon pathfinding (Hevner, 2007). Clinically malformations of cortical development are significant causes of mental retardation, seizures, cerebral palsy, and neuropsychiatric disorders (Barkovich et al., 2005; Guerrini and Marini, 2006; Sarnat and Flores-Sarnat, 2004). Minicolumns are considered a basic architectonic and functional unit of the human neocortex (Buxhoeveden and Casanova, 2002; Casanova et al., 2002). Increased neuron density by 23%, reduced size of neurons in minicolumns, and a concomitant increase in the total number of minicolumns appears to illustrate the bias of local rather than global information processing (Casanova et al., 2002, 2006), resulting in a "hyper-specific brain" (McClelland, 2000). Synchronization of interactions requiring the involvement of distant brain regions is impaired in autism as a result of developmental connectivity deficits (underconnectivity) of smaller neurons (Just et al., 2004; Koshino et al., 2005; Zilbovicius et al., 1995). Structural imaging studies also suggest the overrepresentation of short association fibers in autism, with a regional increase in the volume of white matter (Herbert et al., 2004) favoring the local information processing observed in autistic subjects (Happe, 1999).

1.5 NEURONAL OXIDATIVE STRESS AND METABOLIC CHANGES

An increasing body of evidence suggests that the abnormal rate of development of neurons and neuronal networks in early infancy is followed by metabolic changes, with signs of oxidative stress, enhanced autophagocytosis, and lipofuscin accumulation, leading to early selective neuronal structural and functional changes.

1.5.1 Oxidative Stress in Autism

Oxidative stress is known to be associated with premature aging of cells and can lead to inflammation, damaged cell membranes, autoimmunity, and cell death. The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and high amounts of unsaturated lipids and iron (Juurlink and Peterson, 1998). The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. The vast majority of energy is used by the neurons (Shulman et al., 2004). Glutathione (GSH) is the most important antioxidant for detoxification and elimination of environmental toxins. Due to the lack of glutathione-producing capacity by neurons, the brain has a limited capacity to detoxify reactive oxygen species (ROS). Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, they are most susceptible to oxidative stress. Antioxidants are required for neuronal survival during the early critical period (Perry et al., 2004). Children are more vulnerable than adults to oxidative stress because of their naturally low glutathione levels from conception through infancy (Erden-Inal et al., 2002; Ono et al., 2001). The risk created by this natural deficit in detoxification capacity in infants is increased by the fact that some environmental factors that induce oxidative stress are found at higher concentrations in developing infants than in their mothers, and accumulate in the placenta.

Accumulating evidence from our and other groups suggests increased oxidative stress in autism (reviewed in Chauhan and Chauhan, 2006). Lipid peroxidation is a chain reaction between polyunsaturated fatty acids and ROS, producing lipid peroxides

and hydrocarbon polymers that are both highly toxic to the cell. We have reported that levels of malondialdehyde (MDA), a marker of lipid peroxidation, are increased in the plasma from children with autism (Chauhan et al., 2004). Other studies on erythrocytes (Zoroglu et al., 2004) and urine samples (Ming et al., 2005) have also indicated increased levels of lipid peroxidation markers in autism, thus confirming an increased oxidative stress in autism. Recent studies with the postmortem brain samples from autism and control subjects have provided further evidence on increased oxidative stress in autism. Increased levels of lipid-derived oxidative protein modifications, i.e., carboxyethylpyrrole and iso[4]levuglandin E_2–protein adducts, and heme-oxygenase-1 (an inducible antioxidant enzyme) have been reported in the autistic brain, primarily in the white matter (Evans et al., 2008). Sajdel-Sulkowska et al. (2008) have reported elevated levels of 3-nitrotyrosine (a specific marker for oxidative damage to proteins) in the cerebella of subjects with autism. In addition, we have observed increased lipid peroxidation in cerebellum and temporal cortex of brain in autism (Chauhan et al., 2009). MDA levels were significantly increased by 124% in the cerebellum, and by 256% in the temporal cortex in autism as compared to control subjects.

1.5.2 Lipofuscin in Autism

Lopez-Hurtado and Prieto (2008) revealed a significant increase in the number of lipofuscin-containing cells in the brain of 7- to 14-year-old autistic subjects (by 69% in area 22, 149% in area 39, and 45% in area 44). The increase in the number of lipofuscin-containing cells was paralleled by neuronal loss and glial proliferation. Lipofuscin accumulation is a component of aging (Brunk and Terman 2002a,b; Brunk et al., 1992; Szweda et al., 2003), the neurodegeneration observed in Alzheimer's (Stojanovic et al., 1994) and Parkinson's diseases (Tórsdóttir et al., 1999), developmental syndromes such as Rett syndrome (Jellinger et al., 1988), and autism (Lopez-Hurtado and Prieto, 2008), and such psychiatric disorders as bipolar affective disorder (Yanik et al., 2004) and schizophrenia (Akyol et al., 2002; Herken et al., 2001).

Lipofuscin is an intralysosomal deposit of products of autophagocytosis and degradation of cytoplasmic components, including mitochondria, which cannot be degraded further or exocytosed. Oxidative stress is considered the factor contributing to lipid and protein damage and degradation, resulting in lipofuscin production and accumulation (Brunk et al., 1992; Sohal and Brunk, 1989). The presence of oxidatively modified proteins and lipids in lipofuscin supports the causative link between enhanced oxidative stress, autophagocytosis, and deposition of products of degradation in the lysosomal pathway and lipofuscin (Brunk and Terman, 2002a,b; Szweda et al., 2003; Terman and Brunk, 2004) and suggests that in autism, abnormal development is associated with early signs of oxidative stress and enhanced degradation and, possibly, turnover of cytoplasmic components.

1.5.3 β-Amyloid Precursor Protein and Intraneuronal Amyloid β in Autism

Sokol et al. (2006) detected signs of overexpression of APP in about 40% of autistic subjects. The levels of secreted APP in plasma in children with severe autistic behavior and aggression were two or more times the levels in children without autism, and up to

fourfold more than in children with mild autism. The trend observed in autistic children, with higher levels of secreted β-APP and nonamyloidogenic secreted β-APP, and lower levels of Aβ 1–40 compared to controls, suggests an increased α-secretase pathway in autism (anabolic nonamyloidogenic APP processing). Enzyme-linked immunosorbent assay (ELISA) study of blood plasma from 25 autistic children 2–4 years of age and 25 age-matched control children revealed significantly increased level of secreted amyloid precursor protein alpha (sAPP-α) in 60% of autistic children (Bailey et al., 2008). Western blotting analysis confirmed higher levels of sAPP-α in autistic children.

Amino-terminally truncated intraneuronal amyloid (Aβ) is present in the neurons of control subjects, and the amount of intraneuronal Aβ increases with age (Wegiel et al., 2007). This author's study of 10 brains of autistic people revealed enhanced intraneuronal accumulation of amino-terminally truncated Aβ in 50% of autistic subjects, including in one 5-year-old child and four adults 20, 23, 52, and 62 years of age. A similar pattern was also found in four examined brains of people with autism and isodicentric chromosome 15 (idic15) (Brown et al., 2008). In idic15, excessive accumulation of intraneuronal Aβ might be related to an extra copy of one of the amyloid precursor protein-binding protein (APBA-2) genes localized on chromosome 15. In many brain regions, Aβ is accumulated in large cytoplasmic granules corresponding to deposits of lipofuscin. Numerous large lipofuscin deposits with very strong Aβ immunoreactivity in the neurons of several children and adults with autism appear to reflect severe metabolic stress affecting all of the neurons in the amygdala, all large neurons in the caudate/putamen, a majority of Purkinje cells, and the neurons in the dentate nucleus and nucleus olivaris, but only about 30%–40% of cortical pyramidal neurons. Accumulation of truncated Aβ appears to be a by-product of enhanced degradation of transmembrane APP. The aggregated intracellular Aβ induces the production of ROS and lipid peroxidation products and ultimately results in leakage of the lysosomal membrane (Glabe, 2001). This process appears to affect many neuronal populations, not only in young and old adults, but also in children diagnosed with autism. A metabolic shift with Aβ accumulation in neurons in these brain areas that are involved in the expression of emotions, stereotypic behaviors, and social deficits, such as the amygdala, hippocampus, some striatal subdivisions, and cerebellum, may contribute to cellular dysfunction and the clinical expression of autism.

1.6 CLINICOPATHOLOGICAL CORRELATIONS

Studies of clinicopathological correlations cover several domains of functional deficits in people with autism, including (a) speech, language, and verbal and nonverbal communication, (b) social deficits and face perception, (c) sensorimotor deficits, and (d) cognitive deficits.

1.6.1 Speech, Language, and Verbal and Nonverbal Communication

Expressive language function of individuals with autism ranges from complete mutism to verbal fluency. Verbal abilities are often accompanied by errors in word meaning (semantics) or language and communicative deficits in social context (social pragmatics) (Rapin, 1996; Stone et al., 1997; Wetherby et al., 1998). Studies of the language-related

neocortex, including Wernicke's area (BA 22, speech recognition), Broca's area (BA 44, speech production) and the gyrus angularis (BA 39, reading) of 7- to 44-year-old autistic and 8- to 56-year-old control individuals, revealed reduced numerical density of neurons by 38% in area 22, and by 24% in area 39 in autistic subjects, as well as an increased numerical density of lipofuscin-containing neurons by 50% in BA 22, and 44% in BA 44. These neuronal changes were paralleled by an increase of numerical density of glial cells in all three examined regions. Lopez-Hurtado and Prieto (2008) hypothesized that structural alteration in one or more of these cortical areas may contribute to the communication impairment observed in autism.

1.6.2 Face Perception

All subjects with ASDs have disturbance of social behavior, including abnormalities in social reciprocity and difficulties in use of eye contact, facial expression, and social motivation. Social functioning includes eye contact, processing of faces, identification of individuals, and monitoring of face expression (Baron-Cohen et al., 1994). Patients with autism reveal deficits in face-processing (Grelotti et al., 2001), perception (Schultz, 2005), and recognition (Joseph and Tanaka, 2003).

The face-processing network includes the visual cortex (BA17), which projects via the inferior occipital gyrus to the fusiform gyrus. Fibers from the fusiform gyrus project to the amygdala, and inferior frontal gyrus and orbital cortex (Fairhall and Ishai, 2007; van Kooten et al., 2008). Functional magnetic resonance imaging (fMRI) identified the fusiform gyrus and other cortical regions as supporting face-processing in control subjects, and hypoactivity of the fusiform gyrus in autistic patients (Bolte et al., 2006; Kanwisher et al., 1999; Pierce et al., 2004). Hypoactivation of the fusiform gyrus is believed to be associated with the failure to make direct eye contact in autism (Dalton et al., 2005). Results of imaging-based fusiform volume estimation are inconsistent. Increased (Waiter et al., 2004) and unchanged (Pierce et al., 2001) volume in both hemispheres and increased fusiform gyrus in the left hemisphere (Herbert et al., 2002) were reported. Morphometric studies of the brain of 7 autistic and 10 control subjects revealed a reduced number of neurons in layers III, V, and VI, and reduced volume of neuronal soma in layers V and VI in the fusiform gyrus. No alterations in Brodman area 17 in these autistic individuals suggest that the input from the visual cortex to the fusiform gyrus is intact. These results indicate the underdevelopment of connections in the fusiform gyrus that may contribute to abnormal face perception in autism (van Kooten et al., 2008).

Bailey et al. (1998) noted abnormalities in cytoarchitectonic organization and neuronal density in the superior frontal cortex and superior temporal gyrus in autism. Neurons in the superior temporal sulcus are sensitive to the angle of gaze (Perrett et al., 1985). Neurons that are attuned to particular facial expressions were found in the inferior and superior temporal lobes (Hasselmo et al., 1989). Cortical areas responsive to faces, facial expressions, and angle of gaze send direct projections to the amygdala (Stefanacci and Amaral, 2000). Pathological changes in the amygdala may play a central role in the dysfunction seen in autism, including disturbed components of social cognition such as attention to and interpretation of facial expressions. fMRI studies show that judging from the expression of another

person's eyes what the other person might be thinking or feeling is associated with activation in the superior temporal gyrus, frontal cortex, and amygdala, whereas in subjects with autism, activation appears in the temporal and frontal cortex but not in the amygdala (Baron-Cohen et al., 1999).

1.6.3 Social Attachment—The Role of the Hypothalamus in Behavioral Deficits

Experimental studies revealed that the hypothalamic nucleus paraventricularis (NPV) and the nucleus supraopticus (NSO), producing oxytocin (OT) and vasopressin (VAS), regulate emotional responses, social attachment, cognitive functions, sleep, and appetite (Barden, 2004; Ehlert et al., 2001; Manaye et al., 2005). OT and VAS are relayed from the human brain into the bloodstream via the posterior pituitary. The presence of receptors for both peptides throughout the forebrain, limbic system, thalamus, brain stem, and spinal cord (Raggenbass, 2001) indicates that hypothalamic neuropeptides modulate the function of many brain regions. Developmental changes in the distribution and expression of receptors suggest that the hypothalamic peptides play a significant role both in brain development and function (Shapiro and Insel, 1989). OT is required for the development of social memory. In OT knockout mice, the loss of social memory could be rescued by OT treatment (Ferguson et al., 2000). VAS is necessary for the formation of social memory and OT for retention of newly formed social memories (Popik and Van Ree, 1992; Popik et al., 1992). OT facilitates the learning of social interactions and the formation of associations that are specifically related to the mother (Nelson and Panksepp, 1996).

The initial product of oxytocin mRNA is a polypeptide containing both nanopeptide OT and neurophysin I, separated by tripeptide glycine-lysine-arginine. The result of enzymatic cleavage are intermediate forms containing 10, 11, or 12 amino acids, collectively referred to as carboxy-extended forms of OT (OT-X), and oxytocin (Gabreels et al., 1998; Gainer et al., 1995; Mitchell et al., 1998; Rao et al., 1992). In 5.8- to 11.5-year-old autistic individuals, reduced plasma OT level, deficits in OT prohormone processing (increase in OT-X), and an increase in the ratio of C-terminal extended forms to OT were found. In control children, nearly all OT-X is metabolized to OT, whereas in autistic children, the immature OT forms serve as the primary circulating molecule in the absence of or in compensation for OT (Green et al., 2001). However, experimental studies show that OT-X is not an effective agonist at OT-sensitive sites (Mitchell et al., 1998). Deficient conversion of OT-X to OT in autism could be the result of alterations in the level of prohormone convertases associated with genetic defects (Cook, 1998; Szatmari et al., 1998). The identification of four single nucleotide polymorphisms located within the OT receptor gene of 195 Chinese autistic subjects indicates that abnormal modulation of the OT receptor results in autism (Wu et al., 2005). OT and VAS are known to play a role in repetitive behaviors. Patients with ASDs show a significant reduction in repetitive behaviors following OT infusion (Hollander et al., 2003). In about 60% of subjects with autism, abnormal sleep patterns are observed. VAS is involved in the control of circadian rhythmicity (Swaab, 1997). VAS enhances aggressiveness, anxiety, stress levels, and the consolidation of fear memory (Bielsky et al., 2004; Griebel et al., 2002; Landgraf and Neumann, 2004). OT decreases anxiety

and stress; facilitates social encounters, maternal care, and the extinction of conditioned avoidance behavior (Bale et al., 2001; Champagne et al., 2001; Windle et al., 1997); reduces activation of the amygdala and modulates fear processing (Kirsch et al., 2005). The presence of abnormal levels of hypothalamic neuropeptides in patients with autism provides strong evidence that an abnormality in OT, VAS and other hypothalamic neuropeptides may have a significant contribution to the behavioral features of autism. However, the morphology and biochemistry of the hypothalamus of autistic subjects remains unknown. The only study of the hypothalamic mammillary body of a 26-year-old autistic man revealed that the cell-packing density was increased by 66% (Bauman and Kemper, 1985).

1.6.4 Sensorimotor Deficits, and Repetitive and Stereotyped Behaviors

In individuals with autism, impairments of gross and fine motor function recognized as hypotonia, limbic apraxia, and motor stereotypes are common findings and are more severe in subjects with lower IQ (Rogers et al., 1996). Hand mannerisms, body rocking, or unusual posturing are reported in 37% to 95% of individuals (Lord, 1995; Rapin, 1996; Rogers et al., 1996). In 42% to 88% of subjects with autism, aberrant sensory processing results in a preoccupation with sensory features of objects, over- or underresponsiveness to environmental stimuli or paradoxical responses to sensory stimuli (Kientz and Dunn, 1997). Sensorimotor deficits may by associated with pathological changes in both the nigrostriatal system (basal ganglia) and the cerebellum (Bailey et al., 1998; Kemper and Bauman, 1998; Ritvo et al., 1986; Saitoh and Courchesne, 1998; Sears et al., 1999). Cerebellar abnormality with a deficit/loss of Purkinje cells (Bailey et al., 1998; Kemper and Bauman, 1993, 1998; Ritvo et al., 1986) has been a common finding. Individuals with autism have been classified as affected by cerebellar hyper- or hypoplasia (Saitoh and Courchesne, 1998). A reduced number of Purkinje cells without significant glial activation and a reduced size of Purkinje cells were noticed in the majority of cerebellar reports (Bailey et al., 1998; Fehlow et al., 1993; Kemper and Bauman, 1993; Lee et al., 2002; Ritvo et al., 1986) in 21 of 29 examined cases (Palmen et al., 2004)

Results of evaluation of the size of the cerebellum using MRI are inconsistent. In several MRI studies, smaller cerebellar hemispheres (Gaffney et al., 1987; Murakami et al., 1989) and vermis (Ciesielski et al., 1997; Courchesne et al., 1988; Hashimoto et al., 1995) were reported. In a study by Piven et al. (1997a), the total cerebellar volume was found to be greater in subjects with autism than in the control group, and the increase was proportional to the increased total brain volume. In the cerebellum, boys with autism had less gray matter, a smaller ratio of gray to white matter, and smaller lobules VI and VII than did controls. Despite the inconsistency of reports characterizing topographic autism-associated vermian hypoplasia (Hashimoto et al., 1993; Kaufmann et al., 2003; Levitt et al., 1999; Piven et al., 1997a; Schaefer et al., 1996), several reports show associations between the size of the vermis and deficits in attention-orienting (Harris et al., 1999; Townsend et al., 1999), stereotypic behavior, and reduced exploration in autism (Pierce and Courchesne, 2001).

The reduced size of the pons, midbrain, and medulla in autism reported by Hashimoto et al. (1992, 1993, 1995) was not confirmed in other studies (Hsu et al., 1991; Piven et al., 1992).

Changes in neurons in the deep cerebellar nuclei were noticed by some authors (Kemper and Bauman, 1998) but not by others (Bailey et al., 1998). Structural MRI shows variable patterns of changes. Volumetry of the cerebellum may show no change, hypoplasia, or hyperplasia. Courchesne et al. (1988) reported selective hypertrophy of lobules VI and VII, but these results were not confirmed in other subjects. In part, the pattern may correspond to the functional status of subjects. In highly functioning subjects with autism, hypoplasia of the cerebellum has not been detected (Holttum et al., 1992).

A decrease in the number of GABAergic Purkinje cells is considered the most consistent neuropathological finding in autism, as it was detected in at least 50% of examined cases (Arin et al., 1991; Bailey et al., 1998). Recent studies indicate that preserved Purkinje cells reveal a 40% decrease in the expression of glutamic acid decarboxylase 67 (GAD67) mRNA in autistic subjects relative to control patients (Yip et al., 2007). Moreover, in autism, the basket cells likely provide increased GABAergic feed-forward inhibition to Purkinje cells. The result may include disruption in the timing of Purkinje cell firing and altered inhibition of the cerebellar nuclei, which could directly affect cerebellocortical output, leading to changes in motor behavior and cognition (Yip et al., 2008).

Repetitive and stereotyped behaviors defined as recurring, nonfunctional activities, or interests that occur regularly and interfere with daily functioning are a defining signs of autism. These behaviors include lower-order repetitive motor behavior, intense circumscribed patterns of interests, and higher-order rituals and compulsions (Gabriels et al., 2005). Several studies implicated the role of basal ganglia and frontostriatal circuitry in the pathophysiology of autism, especially in repetitive and stereotyped behaviors. Increased volume of the basal ganglia was reported in several MRI studies (Herbert et al., 2003; Hollander et al., 2005; Langen et al., 2007; Sears et al., 1999). Sears et al. (1999) and Hollander et al. (2005) observed a positive correlation between caudate volumes and repetitive behavior scores. A significant increase in caudate nucleus volume, disproportional to brain volume, was detected in MRI studies in two independent samples of medication-naive subjects with autism (21 high-functioning children and adolescents, and 21 typically developing subjects; 21 high-functioning adolescents and young adults, and 21 healthy subjects) (Langen et al., 2007). Our studies showing a significantly smaller size of neurons in the caudate, putamen, and nucleus accumbens, especially in the brains of children 4–7 years of age suggest a developmental delay in the growth of neurons in the basal ganglia of autistic subjects, which may contribute to basal ganglia dysfunction (Wegiel et al., 2008). MRI and postmortem morphometric studies support the hypothesis that developmental abnormalities in frontostriatal circuitry contribute to repetitive and stereotyped behaviors, which are one of three defining symptoms of autism.

1.6.5 Cognitive Deficits

Many individuals with autism demonstrate a particular pattern on intellectual tests that is characteristic of autism. Performance IQ is usually higher than verbal IQ, and block design is the highest subtest, whereas comprehension is usually the lowest (Siegel et al., 1996). Individuals with autism have poorer adaptive function than would be predicted by IQ alone (Volkmar et al., 1993).

Cognitive deficits might be related in part to the memory system and limbic region abnormalities. Reduced volume of both the hippocampal formation and amygdala were noticed in subjects examined by Aylward et al. (1999), but not in populations examined by other researchers (Piven et al., 1998). Neurons in the hippocampus have reduced complexity of dendritic arbors. They are smaller and more densely packed in various portions of the hippocampal formation, entorhinal cortex, medial nuclei of the amygdala, medial septal nucleus, mammillary nuclei, and anterior cingulate gyrus (Bauman and Kemper, 1985; Kemper and Bauman, 1993). Haznedar et al. (1997) observed reduced volume of the anterior cingulate gyrus and decreased positron emission tomography (PET) activity in subjects with autism. Because the cerebellum is involved in a variety of cognitive and affective processes, abnormalities of both the limbic system and the cerebellum may be linked to the core social and communicative deficits in autism.

The caudate nucleus is an integral component of the frontostriatal network involved in cognitive functions (Chow and Cummings, 1999; Voelbel et al., 2006), including learning (Poldrack et al., 1999), short- and long-term memory (Fuh and Wang, 1995), and planning and problem-solving (Mendez et al., 1989; Schmidtke et al., 2002). The increased volume of the caudate observed in autistic children may be indicative of impaired neuronal pruning, contributing to a decrease in executive function (Voelbel et al., 2006).

1.6.6 Epilepsy-Associated Pathology

The 1% prevalence of epilepsy in the general population increases to 8% in DS, 10% in AD (Menendez, 2005; Risse et al., 1990; Velez and Selwa, 2003), and 33% in autism (Tuchman and Rapin, 2002). The interpretation of developmental changes in autism has been challenged by the need to differentiate among lesions that are not associated with epilepsy, that cause epilepsy, and that are produced by epilepsy (Sutula and Pitkanen, 2001). Recent studies support the hypothesis that epilepsy induces brain alterations that contribute to changes in circuitry, which potentiates the seizure-genic focus (Armstrong, 2005).

Studies of nonautistic subjects indicate that epilepsy-associated pathology includes patchy or laminar neuronal loss and gliosis in the cerebral cortex in one or both hemispheres. In temporal epilepsy, abnormalities were reported in 75% of the specimens examined, and hippocampal sclerosis was found in 50% (Bruton, 1988). Loss of hippocampal neurons correlates with the frequency of tonic-cloning seizures and the total duration of epilepsy (Dam, 1980; Tasch et al., 1999). Loss is accentuated in the CA4 sector and is observed in the granule cell layer in the dentate gyrus. Dispersion of dentate gyrus granular neurons might be a result of seizure-related, disturbed migration of neurons (Bengzon et al., 1997), or epilepsy-enhanced neurogenesis (Ericksson et al., 1998). Ammon horn sclerosis is a progressive lesion that can be induced and propagated by seizures (Armstrong et al., 2005). AQ4

In nearly all cases with hippocampal pathology, changes are also observed in other brain regions. In about 25%, the amygdala, thalamus and mammillary body are affected with neuronal loss. More severe neuronal loss and gliosis in the hippocampus is paralleled by severe neuronal loss and gliosis in the lateral nucleus in the amygdala (Bruton, 1988; Hudson et al., 1993; Thom et al., 1999). Ectopias

with more than 8 neurons per 2 sq. mm of white matter occurred in 43% of epileptic patients but in none of the controls (Hardiman et al., 1988). In 45% of severely affected epileptics, significant neuronal loss and astrocytosis spreading out into the overlying molecular layer is observed in the cerebellar cortex. The severity of the cerebellar damage may range from gross atrophy of most or many folia to the restricted neuronal loss in some folia, especially at their basal portion (Gessaga and Urich, 1985).

Central apnea, asphyxia, and pulmonary edema occurring during a seizure (Nashef et al., 1996) as well as life-threatening cardiac arrhythmias during seizures (Earnest et al., 1992; Jallon, 1997; Nashef et al., 1996; Reeves et al., 1996; Saussu et al., 1998) have been suggested as possible causes of sudden unexpected death in epilepsy (Thom et al., 1999).

Enhanced electric activity of neurons and/or increased cell synaptic transmission with enhanced vesicle exocytosis, both in normal and in disease-affected brains are a common cause of modifications of APP processing and Aβ levels. Epilepsy is associated with an elevation of APP expression (Sheng et al., 1994) and occurs in 10 of 11 examined subjects with diffuse nonfibrillar Aβ plaque formation (mean age 47.9 ± 8.8 years of age) (Mackenzie and Miller, 1994; Mackenzie et al., 1996).

1.7 MECHANISMS AFFECTING BRAIN DEVELOPMENT

1.7.1 BDNF and Neurotrophins in Autism

The neurotrophins, a related family of growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins (NT) NT-3 and NT-4/5, have a major role in the survival, growth, and differentiation of neurons (Conner et al., 1997). During typical brain development, only neurons making the appropriate connections survive and form synapses, whereas neurons that fail to obtain adequate neurotrophins die (Oppenheim, 1991). BDNF is broadly distributed throughout the human central nervous system (CNS) and provides neurotrophic support for many neuronal populations in the cortex, amygdala, hippocampus, and striatum (Murer et al., 2001; Schmidt-Kastner et al., 1996; Tapia-Arancibia et al., 2004). The hypothalamus is the brain structure that contains the highest BDNF protein levels (Katoh-Semba et al., 1997; Nawa et al., 1995; Yan et al., 1997) and BDNF mRNA (Castren et al., 1995; Kawamoto et al., 1996; Yan et al., 1997). In the cerebellum, immunoreactivity was observed in Purkinje cells and the olivary complex of the nuclei (Kawamoto et al., 1996; Murer et al., 2001).

In the basal forebrain of autistic individuals, the level of BDNF was three times higher than in controls (Perry et al., 2001). Miyazaki et al. (2004) observed a higher level of BDNF in the blood samples of young children with autism than in adult control subjects. The mean BDNF levels in sera of children diagnosed with autism and childhood disintegrative disorder were about four times higher than in control children (Connolly et al., 2006). Children with autism and childhood disintegrative disorder have both elevated BDNF levels and levels of autoantibodies against BDNF. Serum BDNF has been shown to be increased after seizures (Binder et al., 2001; Chavko et al., 2002).

1.7.2 Brain Stem and the Role of Serotonin in Brain Development and Clinical Features of Autism

Because 5-hydroxytryptamine (5-HT; serotonin) serves as both a neurotransmitter and an important developmental signal in the brain, dysregulation of the 5-HT system during development may be responsible for many of the abnormalities seen in autism (Whitaker-Azmitia, 2005). In fact, all known chemical inducers of autism including cocaine, thalidomide, valproate, and alcohol modulate 5-HT levels in the brain (Harris et al., 1995; Kramer et al., 1994; Narita et al., 2002; Rathbun and Druse, 1985; Stromland et al., 1994; Williams et al., 2001). A high proportion of children with autism exhibit elevated blood 5-HT levels (hyperserotonemia) and specific alterations in 5-HT biosynthesis. The severity of hyperserotonemia is correlated with the severity of autistic behaviors (Chandana et al., 2005; Chugani et al., 1999; Kuperman et al., 1987). A causal role for serotonergic abnormalities in the etiology of autism is also suggested by studies indicating autism-specific genetic polymorphisms in 5-HT metabolizing enzyme, transporter, or receptor genes (Cohen et al., 2003; Sutcliffe et al., 2005). Also, gender-specific differences in serotonergic regulation during development (Chandana et al., 2005; Chugani et al., 1999), combined with a 52% higher rate of 5-HT biosynthesis in the male than female brain (Nishizawa et al., 1997), and the increased susceptibility of males to early insults imposed by elevated levels of 5-HT (Johns et al., 2002), may contribute to the fourfold higher propensity of males to develop autism compared to females.

As a result of the regulatory role of serotonin affecting the size of neurons, the size of dendritic tree and the number of synapses in innervated cortical and subcortical structures and cerebellum, developmental abnormalities in the serotonergic system may contribute to structural and functional changes in target brain regions and structures. Virtually all regions of the brain receive serotonergic afferents from raphe system neurons. The rostral raphe nuclei form ascending pathways of axons mainly to the forebrain. The caudal raphe system innervates the lower brain stem and the spinal cord (Aitken and Törk, 1988; Lidov and Molliver, 1982). The functions of serotonin are mediated by 14 subtypes of 5-HT receptors in the nervous system (Hoyer et al., 1994; Martin and Humphrey, 1994; Saudou and Hen, 1994a,b). The serotonin$_{2A}$ (5-HT$_{2A}$) receptor is known to be one of the major subtypes and is associated with psychological and mental events (Roth, 1994). The 5-HT$_{2A}$ receptor plays a role in facilitating the formation and maintenance of synapses (Niitsu et al., 1995). Staining for 5-HT$_{2A}$ shows the entire somata and dendritic tree of Purkinje cells in a rat cerebellum (Maeshima et al., 1998). In vitro studies have shown that that 5-HT inhibits the growth and arborization of Purkinje cell dendrites through 5-HT$_{2A}$ receptors and stimulates them through the 5-HT$_{1A}$ receptor (Kondoh et al., 2004). 5-HT promotes the formation of synapses in developing and mature brain and spinal cord (Chen et al., 1997; Niitsu et al., 1995; Okado et al., 1993), and this process is mediated by the 5-HT$_{2A}$ receptor in the spinal cord (Niitsu et al., 1995). Biochemical studies support the hypothesis that developmental defects of the raphe nuclei may make a major contribution to the structural and functional defects of cortical and subcortical structures. However, raphe nuclei have not yet been examined in autistic subjects.

1.8 CLOSING REMARKS

The detected brain structure–specific patterns of structural aberrations in a majority of examined anatomic subdivisions in autism brain may contribute to deficits in expression of emotions, processing of social stimuli, learning of social behaviors, verbal and nonverbal communication, and stereotypic behaviors. Pathological acceleration of brain growth and immaturity of neurons and neuronal networks in early childhood indicate that (a) a significant portion of structural/functional defects starts in early infancy and (b) causative factors dysregulate the mechanisms controlling brain/neuron development. The deceleration of brain growth in the second year of life and the increase of neuronal size in late childhood/adulthood suggests delayed activation of correcting mechanisms. However, the delayed correction of brain and neuronal size does not result in functional recovery. Analysis of the detected pattern of abnormal brain development in autism indicates that early diagnosis and early treatment may prevent or reduce developmental delay, reduce/eliminate secondary structural and functional changes, and improve clinical status throughout the life span.

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ORIGINAL PAPER

The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes

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Abstract Autism is characterized by a broad spectrum of clinical manifestations including qualitative impairments in social interactions and communication, and repetitive and stereotyped patterns of behavior. Abnormal acceleration of brain growth in early childhood, signs of slower growth of neurons, and minicolumn developmental abnormalities suggest multiregional alterations. The aim of this study was to detect the patterns of focal qualitative developmental defects and to identify brain regions that are prone to developmental alterations in autism. Formalin-fixed brain hemispheres of 13 autistic (4–60 years of age) and 14 age-matched control subjects were embedded in celloidin and cut into 200-μm-thick coronal sections, which were stained with cresyl violet and used for neuropathological evaluation. Thickening of the subependymal cell layer in two brains and subependymal nodular dysplasia in one brain is indicative of active neurogenesis in two autistic children. Subcortical, periventricular, hippocampal and cerebellar heterotopias detected in the brains of four autistic subjects (31%) reflect abnormal neuronal migration. Multifocal cerebral dysplasia resulted in local distortion of the cyto-architecture of the neocortex in four brains (31%), of the entorhinal cortex in two brains (15%), of the cornu Ammonis in four brains and of the dentate gyrus in two brains. Cerebellar flocculonodular dysplasia detected in six subjects (46%), focal dysplasia in the vermis in one case, and hypoplasia in one subject indicate local failure of cerebellar development in 62% of autistic subjects. Detection of flocculonodular dysplasia in only one control subject and of a broad spectrum of focal qualitative neuropathological developmental changes in 12 of 13 examined brains of autistic subjects (92%) reflects multiregional dysregulation

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of neurogenesis, neuronal migration and maturation in autism, which may contribute to the heterogeneity of the clinical phenotype.

Keywords Autism · Developmental neuropathology · Subependymal nodular dysplasia · Heterotopia · Dysplasia

Introduction

Autism is characterized by a broad spectrum of clinical manifestations, including (a) qualitative impairments in reciprocal social interactions, (b) qualitative impairments in verbal and nonverbal communication, (c) restricted repetitive and stereotyped patterns of behavior, interests and activities and (d) onset prior to the age of 3 years [1]. In most cases, the etiology is unknown, and patients are diagnosed with idiopathic or non-syndromic autism [10, 43]. About 70% of individuals with idiopathic autism have essential autism, defined by the absence of physical abnormalities, but in 30%, complex autism with dysmorphic features such as microcephaly and/or a structural brain malformation is diagnosed [79]. In 5–10% of cases, autism is diagnosed in association with other disorders such as fragile X syndrome, Rett syndrome, Down syndrome, and tuberous sclerosis [94, 105]. Intellectual impairments, defined as intelligence quotient (IQ) scores less than 70, were reported in 44.6% of children diagnosed with autism [28]. Epilepsy is observed in up to 33% of individuals with autism [106].

The phenotypic heterogeneity is a major obstacle in all areas of autism research [83] and may be the result of a contribution of non-overlapping gene effects. The genetic fractionation of social impairment, communication difficulties and rigid and repetitive behaviors suggests that different features of autism are caused by different genes associated with different brain regions and are related to different cognitive impairments and functional abnormalities [48].

In spite of the broad spectrum of clinical manifestations and striking inter-individual differences, studies of thousands of children have resulted in establishing the clinical diagnostic criteria of pervasive developmental disorder [1]; however, corresponding neuropathological diagnostic criteria do not exist. One of the reasons for the disproportionate progress in clinical and neuropathological studies is the limited tissue resources available for postmortem studies. Between 1980 and 2003, only 58 brains of individuals with autism were examined [85]. Due to the diversity of research aims, of protocols for tissue preservation and of methods of sampling and examination, and the small number of brains examined in an individual project, the pattern of neuropathological changes emerging from these studies remains incomplete and inconsistent.

The hypothesis that autism is associated with neuropathological changes was explored in the first reports published between 1980 and 1993 [7, 21, 22, 27, 42, 50, 51, 82, 90]. Since then, implementation of broader diagnostic terms such as Autism Spectrum Disorder (ASD), examination of larger cohorts, applications of stereology, and functional and structural magnetic resonance imaging (MRI) have resulted in the detection of several major types of pathology, most likely contributing to the clinical phenotype. An emerging concept of autism-related brain pathology integrates evidence of (a) abnormal acceleration of brain growth in early childhood [89], (b) minicolumn pathology [13, 14], (c) curtailed neuronal development [7, 108] and brain structure-specific delays of neuronal growth [111] with indications of abnormalities in brain cytoarchitecture [4, 7], metabolic modifications with abnormal amyloid protein precursor (APP) processing [5, 101], enhanced oxidative stress [17] and enhanced turnover of cell organelles with pigment accumulation and glial activation [68].

In spite of the conceptual limitations, "localizing" models are still the main approach to the identification of pathological changes as a component of the networks' structural and functional abnormalities [81]. We hypothesize that dysregulation of neurogenesis, neuronal migration and maturation is also reflected in qualitative, focal, developmental alterations of brain microarchitecture. The aim of this study is to detect the pattern of focal, qualitative, developmental defects in autism brain, including their type, topography and severity, and to identify the structures and brain regions that are prone to developmental alterations in autism.

Materials and methods

The autistic cohort study consisted of 13 subjects (4–62 years of age), including 9 males (69%) and 4 females (31%), while the control cohort consisted of 14 subjects (4–64 years of age), including 9 males and 5 females (Table 1).

Clinical and genetic characteristics of the autistic subjects

The source of our clinical data was the medical records of the autistic subjects, which consisted of psychological, behavioral, neurological and psychiatric evaluation reports. All of the records were obtained after the subjects' deaths. The Autism Diagnostic Interview-Revised (ADI-R) was administered to each donor family as a standardized assessment tool in order to confirm the diagnosis on a postmortem basis. Inclusion of the subject in this study was based on a summary of scores of four domains:

Table 1 Material examined

#	Group	Brain bank #	Sex	Age (years)	Cause of death	PMI (h)	H	Brain weight (g)
1	A	IBR425-02	M	4	Drowning	30	R	1,280
2	A	UMB-1627	F	5	Traumatic multiple injuries	13.2	R	1,390
3	A	B-6403	M	7	Drowning	25	R	1,610
4	A	B-5666	M	8	Rhabdomyosarcoma	22.2	R	1,570
5	A	B-5342	F	11	Seizure-related drowning	12.9	L	1,460
6	A	B-5535	M	13	Seizure-related	8	L	1,470
7	A	B-6115	F	17	Cardiac arrest related to cardiomyopathy	25	L	1,580
8	A	UMB-1638	F	21	Seizure-related respiratory failure	50	R	1,108
9	A	B-6337	M	22	Seizure-related	25	R	1,375
10	A	IBR93-01	M	23	Status epilepticus-related respiratory failure	14	R	1,610
11	A	B-6212	M	36	Cardiac arrest	24	R	1,480
12	A	B-6276	M	56	Cardiac arrest	3.35	R	1,570
13	A	B-7090	M	60	Pancreatic cancer	26.5	R	1,210
1	C	B-6736	F	4	Acute bronchopneumonia	17	R	1,530
2	C	UMB-1499	F	4	Lymphocytic myocarditis	21	R	1,222
3	C	UMB-4898	M	7	Drowning	12	R	1,240
4	C	UMB-1708	F	8	Traumatic multiple injuries	20	R	1,222
5	C	BTB-3638	M	14	Electrocution	20	R	1,464
6	C	UMB-1843	F	15	Traumatic multiple injuries	9	R	1,250
7	C	UMB-1846	F	20	Traumatic multiple injuries	9	R	1,340
8	C	UMB-1646	M	23	Ruptured spleen	6	R	1,520
9	C	UMB-4543	M	29	Traumatic multiple injuries	13	R	1,514
10	C	UMB-1576	M	32	Traumatic compressional asphyxia	24	R	1,364
11	C	BTB-3899	M	48	Atherosclerotic heart disease	24	L	1,412
12	C	IBR252-02	M	51	Myocardial infarct	18	L	1,450
13	C	BTB-3983	M	52	Heart atherosclerosis	13	R	1,430
14	C	B-6874	M	64	Cardiac arrest	28	R	1,250

PMI postmortem interval, *H* hemisphere, *R* right, *L* left

(a) qualitative abnormalities in reciprocal social interaction; (b) qualitative abnormalities in verbal and nonverbal communication; (c) restricted, repetitive and stereotyped patterns of behavior; and (d) abnormality of development evident at or before 36 months [69]. All 13 autistic subjects met ADI-R criteria for autism. For some subjects, the intellectual evaluation was available and was based on the Wechsler Intelligence Scale for Children III and the Woodcock-Johnson Tests of Achievement-Revised (Table 2). Eight subjects were diagnosed with intellectual disability, usually in the range from mild to severe (61%). Six of 13 autistic subjects had seizures (46%). In five cases, the age of onset of seizures was from 14 months to 5 years of age. A 23-year-old autistic male had only one seizure, which was reported as the cause of his death. In one child, an abnormal EEG was detected, but without seizures.

Several forms of challenging behaviors and behavioral disorders were noted, including self-injurious behavior (six cases, 46%), aggression (four cases, 31%), hyperactivity (three cases, 23%), obsessive compulsive disorder (two cases, 12%) and depression and mania (a single case of each).

For three of the 13 autistic subjects, the list of high-confidence copy number variations identified both by quantiSNAP and Partek HMM computational algorithm was posted on the ATP portal by Drs. Steve Scherer and Richard Wintle from The Center for Applied Genomics, Toronto. The copy number variations detected in the three autistic subjects do not differ from those commonly observed [75], except for the loss of 25,505 kb within Neuropeptide S Receptor 1 (NPSR1) gene at 7p15–p14 detected in a 22-year-old autistic male (B-6337). NPSR1 has not been linked to autism in the genomic reports [103, 112]; however, an association of NPSR1 copy number variation with allergies has been reported [11] that might be linked to the patient's history of allergies.

Originally, 38 brains, including 20 brains of autistic and 18 brains of control subjects, were assigned to this project. However, application of the clinical and neuropathological

Table 2 Behavioral and neurological signs, and the type and topography of developmental abnormalities

Brain bank #	Psychiatric disorders and neurological symptoms	Mental retardation (MR)	Seizures age of onset	Type and topography of developmental abnormalities
IBR425-02	Hyperactivity. Tantrums. Self-injurious behavior	–	–	No changes
UMB-1627	Aggression	–	–	Focal neuronal heterotopia in white matter of the anterior cingulate gyrus
B-6403	–	–	14 months	Subependymal nodular dysplasia in the wall of the occipital horn of the lateral ventricle. Two periventricular nodular heterotopias (2 and 4 mm in diameter) near the frontal horn of the lateral ventricle. Tuber-like expansion of the tail of caudate nucleus into the lumen of the ventricle. Flocculonodular dysplasia
B-5666	–	–	Abnormal EEG; no seizures	Cortical dysplasia in the middle and inferior temporal gyri with focal dyslamination, clustering of dystrophic neurons and severe local neuronal deficits. Several focal dysplastic changes within CA. Flocculonodular dysplasia affecting almost entire lobe
B-5342	Pervasive developmental disorder. Hyperlexia	Mild MR	4.5 months	Focal cortical dysplasia. Dysplasia of the granule layer of the dentate gyrus. Subcortical heterotopia in the inferior frontal gyrus. Heterotopia in vermis and in cerebellar white matter
B-5535	Hyperactivity. Self-injurious behavior including head-banging	Moderate to severe MR	2 years	Thickening of the subependymal cell layer. Focal dysplasia within CA1 pyramidal layer with neuronal deficit, abnormal neuron morphology and spatial orientation. Multifocal dysplasia of the dentate gyrus with distortion of the shape of granule and molecular cell layers. Focal dysplasia within vermis
B-6115	Sensory integration disorder	–	–	Flocculonodular dysplasia affecting the majority of lobe volume. Cortical angioma
UMB-1638	ADHD	Moderate MR	5 years	Focal dysplasia within CA1 with diffuse neuronal deficit but without glial activation
B-6337	Obsessive compulsive disorder. Mania. Tourette syndrome. Self-injurious behavior	MR	–	Minor focal flocculonodular dysplasia
IBR93-01	Hyperactivity. Aggressive and self-injurious behavior	Severe MR	23 years	Focal dysplasia within islands in the entorhinal cortex. Pineal gland cysts
B-6212	Obsessive compulsive disorder. Depression, aggression, and anxiety	Severe MR	–	Several areas of focal cortical dysplasia within frontal cortex and insula with local loss of vertical and horizontal organization. Merger of ventral portion of the claustrum with insula. Flocculonodular dysplasia
B-6276	Aggression and self-injurious behavior, anxiety and agitation	Moderate MR	–	Focal dysplasia within CA1 sector with focal neuronal deficit. Heterotopia within stratum oriens. Flocculonodular dysplasia affecting approximately 70% of the lobe
B-7090	Disturbed movement coordination (walking like drunk)	MR	3 years	Three focal dysplasias in the frontal cortex. Dysplasia of layers 1–3 in the entorhinal cortex with missing numerous islands of the stellate neurons. Severe hypoplasia of cerebellar lobes 1–4. Reduced convolutions within dentate nucleus

Developmental abnormalities in brains of autistic subjects

exclusion criteria reduced the size of the cohort to 27 brains. Based on the results of the ADI-R, two cases were excluded, including one case diagnosed with atypical autism, and one that did not meet ADI-R criteria. Based on postmortem evaluation, five more autistic cases were excluded: one due to severe postmortem autolytic changes, three due to severe global hypoxic encephalopathy related to the mechanism of death, and one due to multiple microinfarcts. Moreover, four brains of control subjects were disqualified due to severe postmortem autolysis. In all these brains, neuronal loss, changes of neuronal size and shape, and gliosis were so severe that they masked and distorted the qualitative and quantitative characteristics of the developmental alterations associated with autism.

Brain tissue preservation

Brains of 13 autistic and 14 age-matched control subjects were examined by postmortem MRI and neuropathologically. The postmortem interval (PMI) varied, ranging from 6 to 27.8 h in the control group (16 h on average; SD 6 h) and from 8 to 30 h in the autistic group (20 h on average; SD 12 h). The median PMI was 15 h.

The brain hemispheres were removed using standard techniques, exercising extra care to avoid damaging the brain tissue. The brain was weighed in the fresh state. The fresh brain was sagittally cut through the corpus callosum and brainstem. Half of the brain was fixed in 10% buffered formalin. Following at least 3 weeks of fixation, the brain hemisphere was scanned using MRI. The aim of the MRI application was to determine the type of developmental changes detectable by MRI and to microscopically characterize MRI findings. All brains within this project were scanned (L.A.S.L.) using a standardized protocol (established and implemented for this and for other postmortem MRI studies by L.A.S.L. and M.L.). MRI scans were acquired on a 1.5 T GE Signa Imager (General Electric, Milwaukee, USA). The research scan consisted of a 124-slice T1-weighted fast gradient echo acquired in a coronal orientation perpendicular to the long axis of the hippocampus with a 1.5-mm slice thickness, which encompassed the entire brain hemisphere without gaps or wrap artifacts (FOV = 25 cm; NEX = 1; matrix = 256 × 192; TR = 35 ms; FA = 60°). All file names were assigned sequential code numbers, and demographic information was removed from image headers [9]. MRI scans were first screened in a diagnosis–blind manner, and the brains with abnormalities were re-evaluated by both radiologists and neuropathologists to determine the topography, type, and size of lesions detected with both methods.

The brain hemisphere was fixed with 10% buffered formalin. Formalin was washed out from the tissue during an overnight tap water rinsing. Brains were dehydrated using a series of increasing ethyl alcohol concentrations (50% ethanol 3 days; 70% ethanol 4 days; 80% ethanol 3 days; 95% ethanol 4 days). The brain hemisphere was embedded in 8% celloidin [53]. During hardening, celloidin blocks were exposed to chloroform vapors for approximately 2.5 weeks, and celloidin blocks were then stored in 70% ethanol. For sectioning, the block was attached to the block holder with 10–15 ml of 8% celloidin. To fasten adhesion of the block to the holder, the block with the holder attached was immersed in 70% ethanol overnight. Serial 200-μm-thick sections were separated with filter paper and stored in 70% ethanol. For the four control and four brains of autistic subjects, alternative series of 200- and 50-μm-thick sections were preserved. To ensure the same probability of detection of changes in each case, every 200-μm-thick section, with a distance 1.2 mm, was used in this project. Sections were washed in water for 2–3 h, stained with cresyl violet (CV) and mounted with Acrytol.

One neuropathologist (I.K.) examined, in a blind-to-diagnosis fashion, on average 120 hemispheric CV-stained sections per case with a 1.2-mm distance between sections. Two-step screening included examination at low magnification (28×) using Zeiss DL2 Documator and microscopic examination using objective lenses from 5× to 100×. Two other neuropathologists (T.W. and J.W.) examined all histological slides for which pathology was detected during the primary screening. The defects of neurogenesis, neuronal migration, and dysplastic changes that they detected were summarized in this report.

Tissue acquisition for this program project is based on individual tissue transfer agreements between the program project's principal investigator and several tissue banks: (a) the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, (b) the Harvard Brain Tissue Resource Center and (c) the Brain Bank for Developmental Disabilities and Aging of the NYS Institute for Basic Research in Developmental Disabilities. Each brain hemisphere number given by the institution that received the donation was used as the only identifier of clinical records and tissue samples. Brain Bank identification of tissue samples is listed in Tables 1 and 2 to keep non-overlapping records of results of examination of brains in different projects and research groups. The Institutional Review Board of the New York State Institute for Basic Research in Developmental Disabilities approved the methods applied in this study.

Results

Neuropathological evaluation of serial coronal hemispheric sections from the cerebral and cerebellar hemispheres of

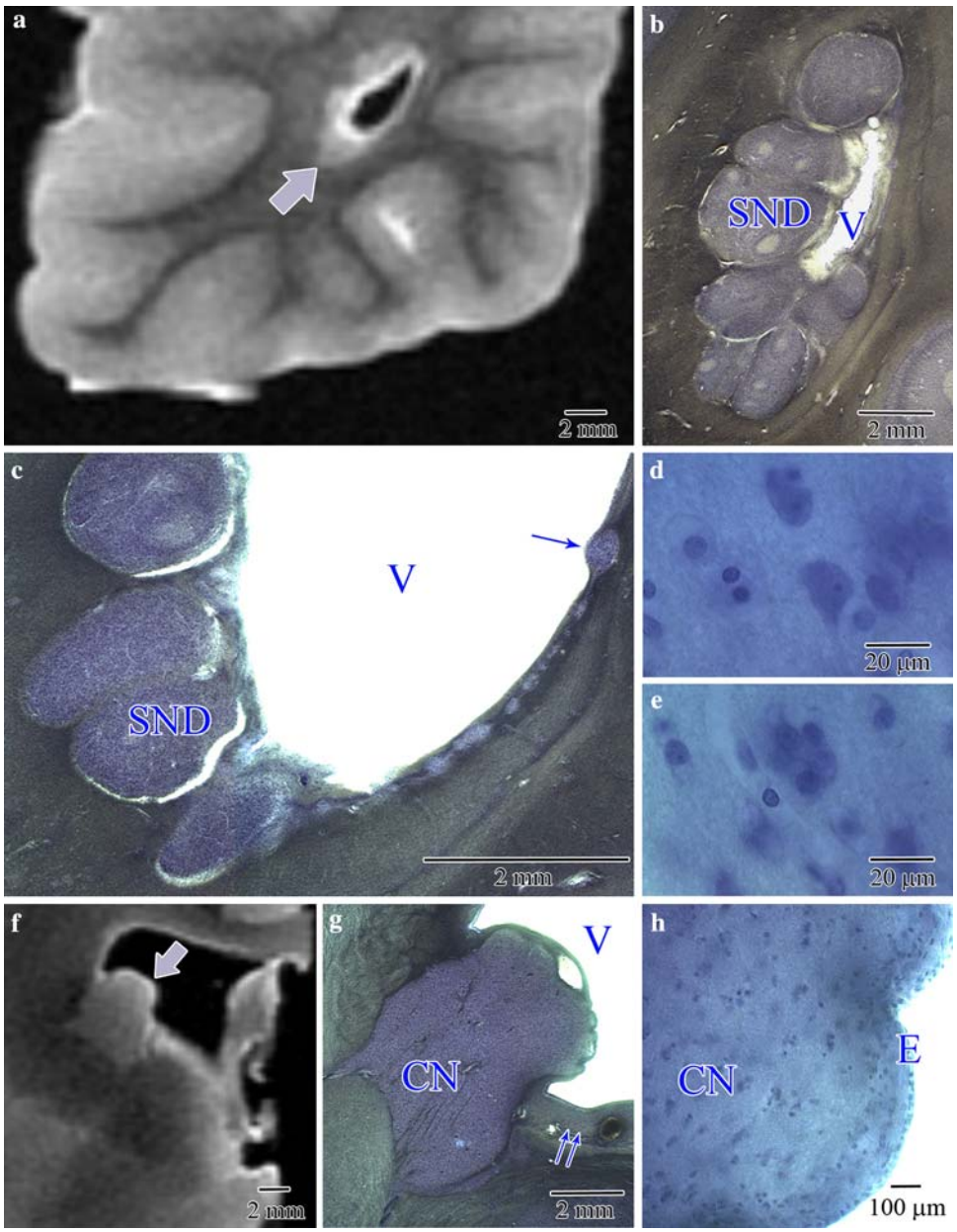

Fig. 1 Nodules in the wall of the lateral ventricle detected in postmortem MRI (**a**) in the brain of a 7-year-old male diagnosed with autism (B-6403) revealed features of subependymal nodular dysplasia (SND; **b**) in examination of CV-stained sections. **c** Numerous large and small nodules (*arrow*) dispersed within subependymal cell layer. They contained a few pyramidal-like neurons (**d**) and numerous poorly differentiated cells (**e**). Tuber-like expansion of the caudate nucleus (*arrow*) into the ventricle lumen is shown in MRI (**f**) and in CV-stained section (**g**). **g** A thick subependymal cell layer above and below (*two arrows*) the caudate nucleus (*CN*), and the absence of matrix in the tuber-like area. Under ependymal (*E*) cap of the caudate nucleus (*CN*) tuber-like expansion, small poorly differentiated neurons are present (**h**)

13 autistic and 14 control subjects revealed more details characterizing the topography and severity of changes than did standard sampling of brains for routine neuropathological evaluation. A broad range of changes was found. Developmental abnormalities included subependymal nodular dysplasia, heterotopia and very common dysplastic changes within the neo- and archicortex, hippocampus and cerebellum in 12 of 13 examined brains of the autistic subjects (92%) (Table 2). The general result of these developmental defects was a multifocal disorganization of gray and white matter. The developmental pathology observed in control brains was limited to one cerebellar dysplasia.

Alterations of the subependymal cell layer and subependymal nodular dysplasia

In two autistic subjects, there was a several-fold local increase in the thickness of the subependymal cell layer. Numerous subependymal nodules were found within a pathologically thickened subependymal cell layer, in the wall of the occipital horn of the lateral ventricle of a 7-year-old male, which reflects a subependymal nodular dysplasia (Fig. 1a–e). Nodules occupied 13.3 mm of the caudal portion of the occipital horn of the lateral ventricle. The diameter of round/oval nodules varied in size from 285 to 3,310 µm. While the smallest nodules were dispersed

within the subependymal cell layer, the large nodules expanded partially into the white matter, and partially into the lumen of the ventricles and were detectable on MRI (Fig. 1a) and CV-stained histological sections (Fig. 1b, c). The effect was narrowing of the ventricle and the tuberous appearance of the ventricular wall. There were large tubers that contained dysplastic neurons with a partially modified morphology of pyramidal, multipolar or bipolar large neurons (Fig. 1d) and irregularly shaped medium and small neurons. Neurons in the small nodules were small and poorly differentiated (Fig. 1e). In the large nodules, several hypocellular areas were observed. The nodules were free of oval or polygonal giant cells or ballooned glial cells, as well as signs of calcification.

In the brain with the subependymal nodular dysplasia, an abnormal tuberous expansion of the caudate nucleus was detected on MRI (Fig. 1f) and in histological sections (Fig. 1g, h). Only the ependyma separated the tuber-like expansion of the caudate from the ventricle lumen. The very thick subependymal cell layer that was present close to the caudate was substituted by loosely arranged and poorly differentiated neurons in the affected area.

Heterotopia

Heterotopias were found in the brains of four autistic subjects and no control subjects. The topography of the lesions was different in each case. Subcortical heterotopias were detected in the white matter of the anterior cingulate gyrus of a 5-year-old (Fig. 2a, b) and in the inferior frontal gyrus in an 11-year-old subject. Periventricular heterotopias were detected near the wall of lateral ventricle in 7-year old subject (Fig. 2c, d). A single heterotopia was noted in the stratum oriens of the hippocampus. In the cerebellum of the 11-year-old subject, heterotopias were detected in the vermis and the cerebellar white matter close to the dentate nucleus (Fig. 2f–h). These defects of migration were observed in two brains as a single aggregate of gray matter, in one brain as two aggregates and in one brain as three lesions measuring from 1 to 3 mm in diameter. Subcortical and periventricular heterotopias comprised poorly differentiated oval or multipolar neurons without spatial orientation (Fig. 2a) or had a distorted laminar organization (Fig. 2e). Cerebellar heterotopias had a distorted morphology of the granule and molecular layers with a few dispersed Purkinje cells (Fig. 2g, h).

Dysplasia within neocortex and archicortex, hippocampus and cerebellum

The multifocal neocortical dysplasia detected in four brains of autistic subjects (31%) was associated with a local loss of vertical and horizontal organization of the neocortex, formation of abnormal layers, loss of orientation of neurons (Fig. 3a, b) and thickening of the affected portion of the cortical ribbon. A focal dysplasia in the entorhinal cortex, observed mainly in the second layer with a local absence of islands and/or reduced number of neurons, was found in the 23-year-old and the 60-year-old autistic subjects (15%) (Fig. 3c, d). A lack of giant multinuclear neurons and large, ballooned glial cells typical of focal cortical dysplasia indicated that the observed developmental changes in neocortex and archicortex reflect a more subtle cortical malformation, classified usually as focal cortical microdysgenesis.

Two types of changes were observed in the dentate gyrus. An abnormal migration of granule neurons into the molecular layer resulted in the formation of an additional fragmentary granule cell layer (Fig. 3e). In other areas, granule cells formed irregular circles and loops (Fig. 3f).

In the CA1 sector of a 13-year-old male, several areas of dysplastic changes with a significant deficit of pyramidal neurons without gliosis were found (Fig. 3g). In affected areas, the size and shape of neurons varied over a wide range. Pyramidal neurons were very rare, whereas small irregular or oval-shaped, poorly differentiated neurons prevailed (Fig. 3h). In the dysplastic area in the CA1 sector of the 56-year-old autistic subject, an opposite trend was present, with thickening of the pyramidal layer and an increased packing of dysplastic neurons (not shown).

The most common developmental abnormality within the cerebellum was dysplasia, which was detected in seven autistic subjects (54%) and in the cerebellum of one control subject.

Flocculonodular dysplasia (Fig. 4a, b), usually affecting the entire nodule, was found in the cerebellum in six autistic subjects (46%). In the dysplastic areas, a thin granule layer formed the labyrinth, which was mixed with irregular islands of the molecular layer. Clusters of granule cells and a few Purkinje cells were dispersed within the distorted molecular layer. The only developmental abnormality detected in the control group was flocculonodular dysplasia in the cerebellum of the 51-year-old control subject (not shown). Local cortical dysplasia was also detected within the vermis of the 13-year-old autistic male. In the affected area, the cytoarchitecture of the molecular and granule layers and the Purkinje cells was completely disorganized (Fig. 4c, d).

In the cerebellum of the 60-year-old autistic male, severe hypoplasia affected lobes 1–4 (Fig. 4e). The thickness of the molecular and granular layer was decreased by almost 50% in comparison to that of the non-affected areas (Fig. 4e, f). The number of Purkinje cells was significantly reduced in the hypoplastic area. Hypoplastic changes within the portion of cerebellar cortex were observed,

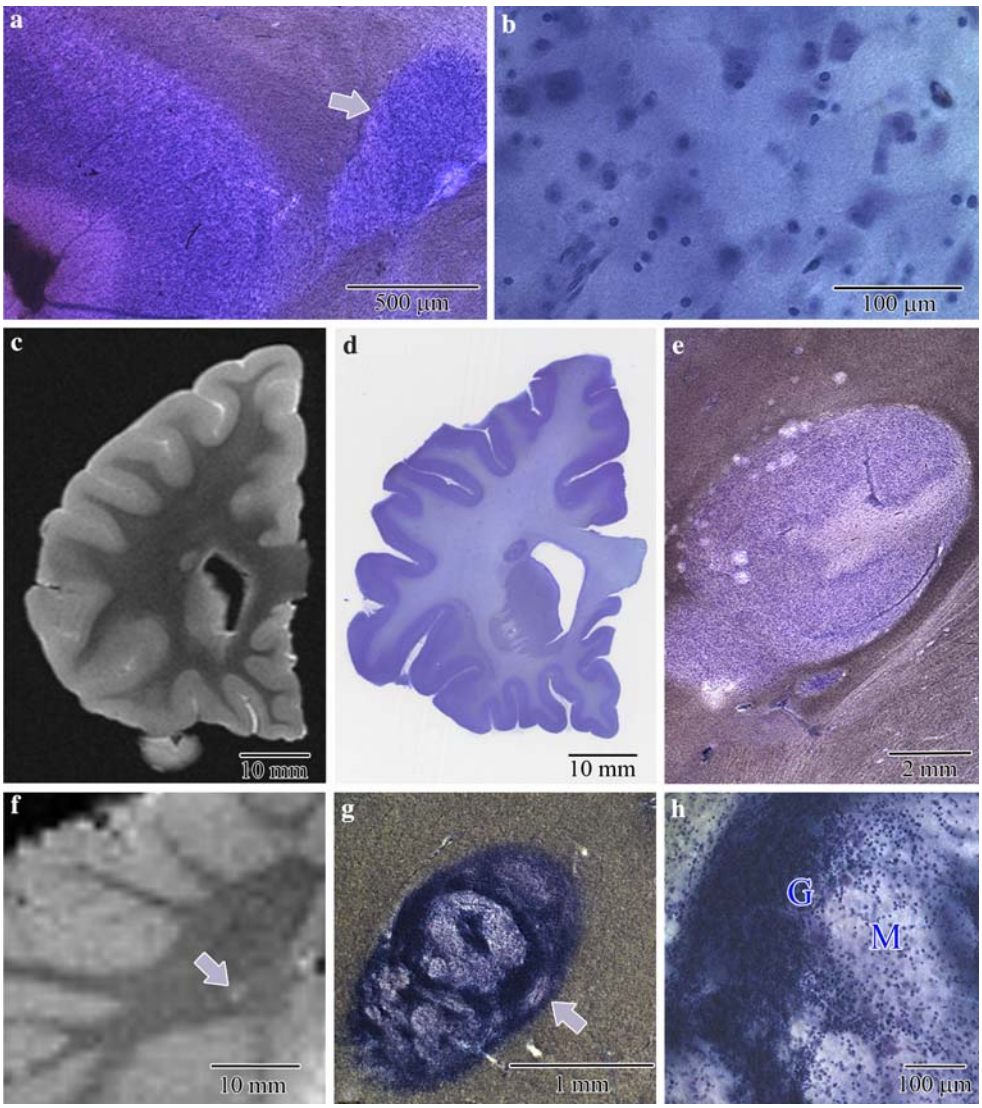

Fig. 2 Large subcortical heterotopia within anterior cingulate gyrus in a 5-year-old autistic child (UMB-1627) (**a**) contained dysplastic neurons without spatial orientation (**b**). Periventricular heterotopia near the frontal horn of the lateral ventricle (**c** MRI, **d**, **e** CV-stained section) shows a structure resembling molecular, granule and pyramidal layers in a 7-year-old autistic subject (B-6403). MRI (**f**), low (**g**) and large (**h**) magnification of heterotopia (*arrow*) with dysplastic granule (*G*) and molecular layer (*M*) detected within cerebellar white matter in an 11-year-old autistic subject (B-5342)

together with a significantly reduced convolution of the dentate nucleus (Fig. 4g).

Discussion

This neuropathological study revealed a broad spectrum of focal developmental abnormalities and pre- and perinatally acquired lesions in 92% of the brains of autistic subjects and striking inter-individual differences in the type and topography of changes. Evidence that different features of autism are caused by different genes associated with different brain regions [48] suggests a link between regional developmental alterations in the brain and different components of the autistic phenotype.

Altered neurogenesis in autism

Increased brain mass in autistic children and some autistic adults [89], increase in the numerical density of neurons [13, 14], reduced size of neurons [7] and brain structure-specific delay of neuronal growth [111] indicate alterations in neuronal and brain growth in autistic individuals. The subventricular zone of the lateral ventricles [26] and the dentate gyrus [33] are active sites of neurogenesis in adult humans. Several of our findings support the hypothesis of

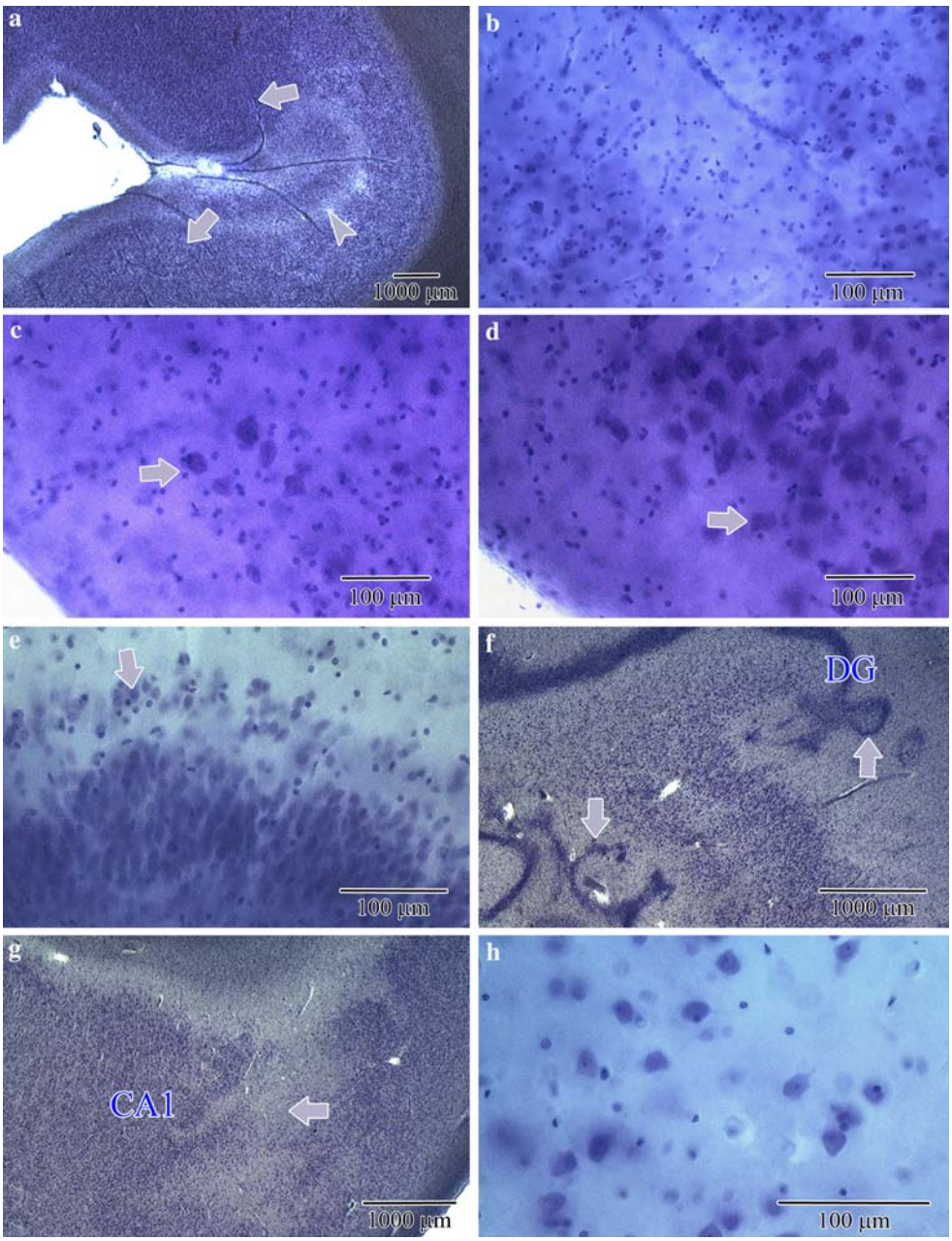

Fig. 3 Dysplastic changes within neocortex (**a**, **b**), entorhinal cortex (**c**, **d**), dentate gyrus (**e**, **f**) and the cornu Ammonis (**g**, **h**). Focal dysplasia in frontal cortex with loss of vertical and horizontal cytoarchitecture (*two arrows*) and abnormal (*arrowhead*) laminar organization (**a**). Dysplastic neurons within affected area (B-6212) (**b**). Microdysgenesis within the entorhinal cortex with deficit of stellate neurons in the islands (**c**) and normal morphology of islands in adjacent cortex (**d**) in 60-year-old autistic subject (B-7090). Microdysgenesis of the dentate gyrus with dispersion of granule cells within the molecular layer (**e**, *arrow*) and distortion of the granule cell layer shape (**f**, *arrows*) in 13-year-old autistic male (B-5535). CA1 sector microdysgenesis with local deficit of pyramidal neurons (**g**, *arrow*) without markers of gliosis but with signs of poor differentiation of dysplastic abnormally arranged neurons (**h**) in 13-year-old autistic subject (B-5535)

altered neurogenesis in autistic subjects. The increased thickness of the subependymal cell layer, subependymal nodular dysplasia, abnormal growth of the dentate nucleus and dysplasia of the granule layer in the dentate gyrus, detected in this study, appear to be signs of abnormal neurogenesis in the brains of three autistic subjects. Subependymal nodules were reported in approximately 80% of patients with tuberous sclerosis, a disorder that is highly associated with epilepsy, autism and mental retardation [73]. Tuberous sclerosis nodules were detected in one fetus [12], suggesting that fetal development of subependymal nodules can lead to the early onset of epilepsy

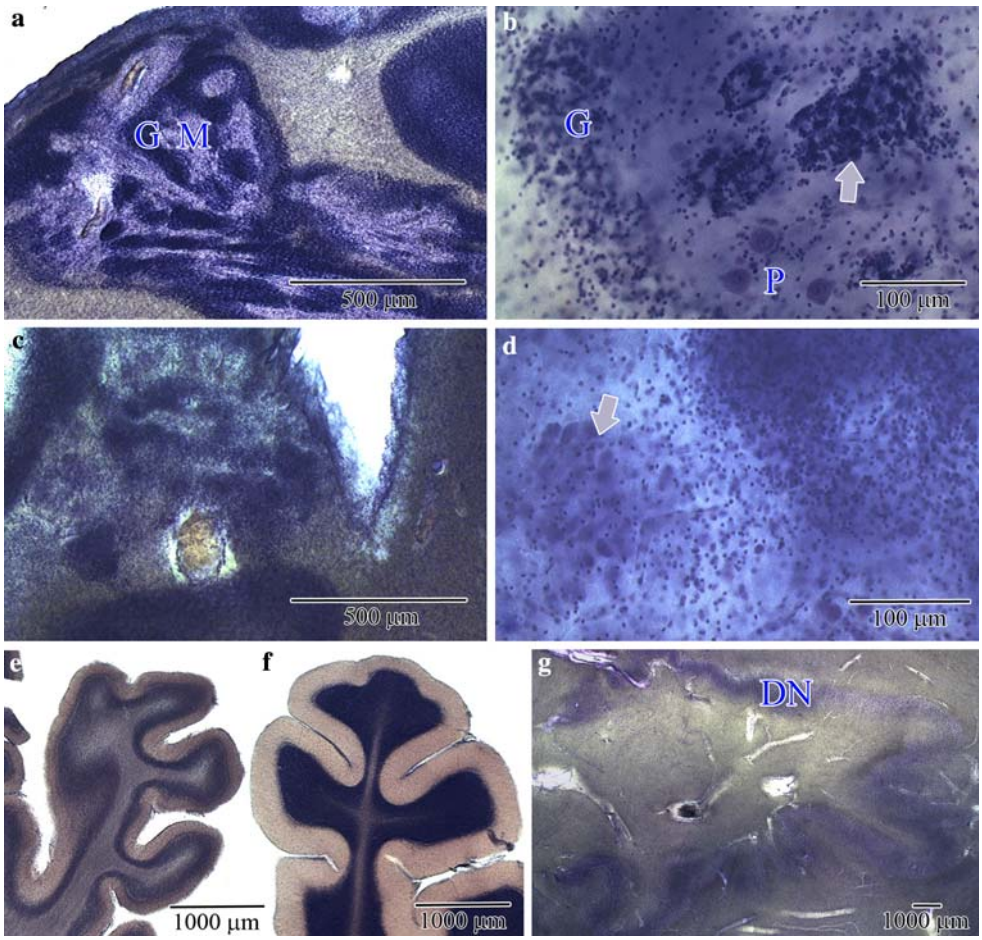

Fig. 4 Flocculonodular dysplasia in cerebellum of 56-year-old autistic subject (B-6276) (**a**) with thin irregular granule (*G*) and molecular (*M*) layer. **b** Dysplastic granule layer (*G*), ectopic granule cells (*arrow*) in the molecular layer, and loosely dispersed Purkinje cells (*P*) (B-6276). Cortical dysplasia within vermis of 13-year-old autistic male (**c**) with dysplastic granule neurons mixed with heterotopic (*arrow*) large cells (**d**) (B-5535). **e** Severe hypoplasia of cerebellar lobe 3 and unmodified lobe 6 (**f**), respectively, within the cerebellum of a 60-year-old autistic male (B-7090). In the affected region, the thickness of the hypoplastic molecular and granule cell layer was reduced by about 50%. Almost half of the dentate nucleus (*DN*) was less convoluted than the unaffected part (**g**)

that was diagnosed at the age of 14 months in a neuropathologically examined autistic male. The subependymal nodules detected in this autistic male's brain are partially similar to tubers seen in subjects diagnosed with tuberous sclerosis [24]. The cause of subependymal nodular dysplasia in the examined subject is unknown. In the reported subjects, bilateral periventricular nodules are linked to mutations of the filamin A (FLNA) gene located on chromosome Xp28. Filamin A is an actin-crosslinking protein that is essential for cell locomotion [16], and nodule formation might be related to a defect in cell migration. The presence of miniature nodules that were built of poorly differentiated small neurons within the subependymal cell layer and an increase in nodular size with signs of growth and differentiation of neurons suggests that neurogenesis, differentiation and maturation of neurons were in progress within the subependymal germinal matrix of the 7-year-old autistic child. This interpretation of subependymal nodule genesis is consistent with lineage studies demonstrating that cells in nodules express cellular markers that are typical for progenitors derived from the subventricular germinal zone [35, 67]. However, in contrast to the subependymal nodules seen in subjects with tuberous sclerosis, in the examined autistic subject, the nodules seen were small (from 258 to 3,310 µm in diameter), and did not have the characteristic ovoid or polygonal giant cells, 80–150 µm in diameter, giant cells with multiple and peripherally displaced nuclei [25], or balloon cells, which are considered the sine qua non histopathological features of the cortical tubers and subependymal nodules observed in tuberous sclerosis [73].

The enlarged caudate nucleus detected in the brain of the 7-year-old autistic subject is consistent with MRI reports documenting an increased volume of basal ganglia, including the caudate, in autism [54, 55, 66, 99]. A disproportionate increase of the caudate nucleus volume [66] suggests that in brains of some autistic individuals, extended neurogenesis within the subependymal cell layer may contribute to abnormal growth of the caudate nucleus. A similar process has been observed in the brains of people with Huntington disease, showing enhanced neurogenesis in the subependymal layer and suggesting renewal of the neuronal population in a degenerating caudate nucleus [26]. The caudate nucleus is a part of the fronto-striatal network involved in several functional domains that are impaired in autism, including lower order repetitive motor

behavior; intense circumscribed patterns of interests and higher order rituals and compulsions [41], and defects in cognitive functions [19, 109], planning and problem-solving skills [78, 98], short- and long-term memory [40] and learning [88].

Defective migration in autism

Heterotopia is a sign of altered migration leading to an abnormal distribution of gray matter nodular masses with disorganized or rudimentary lamination within the periventricular area (periventricular heterotopia) or subcortical white matter (subcortical heterotopia) [2]. In the examined cohorts, heterotopias were detected in the brains of four autistic subjects and in the brain of one control subject. Heterotopias are associated with mutations in the filamin 1 gene (FLNA1) [39, 46] and the chromosome X-linked DCX gene that codes for doublecortin, a protein expressed during brain development in migrating neurons, and in the cortical plate [29, 44, 45], which is involved in the formation of the microtubules necessary for neuronal migration [15]. Periventricular nodular heterotopia has been reported to be associated with pharmaco-resistant seizures in 80–90% of patients [31]. In the examined cohort, two periventricular heterotopias were detected in the brain of a child with subependymal nodular dysplasia and seizures diagnosed at 14 months of age (B-6403). Early onset epilepsy, diagnosed at the age of 4.5 months, might be related to the multiple heterotopias found within the frontal inferior gyrus, vermis and cerebellar white matter, coexisting with a focal cortical dysplasia and dentate gyrus dysplasia (B-5342).

Cortical, hippocampal and cerebellar dysplasia in autism

The most common form of developmental changes detected in the examined brains was focal dysplasia, which was observed in 11 (85%) of the autistic subjects. The morphology of focal dysplasias appears to reflect signs of abnormal migration, neuronal immaturity and altered cell arrangement, resulting in focal distortion of cytoarchitecture. In spite of similarities, the dysplastic changes in the neocortex and archicortex, dentate gyrus and cornu Ammonis and cerebellum also reveal a brain structure-specific pattern of dysplastic changes in autism.

Dysplasias encompass a spectrum of changes ranging from a mild form of cortical disruption, without cellular abnormalities, to the most severe form with cortical dyslamination, with abnormal morphology of neurons and astrocytes [93, 96, 107]. Focal cortical dysplasias with giant neurons and balloon cells [107, 113] are histopathologically similar to tubers containing giant cells in tuberous sclerosis complex [25, 73], suggesting a common pathogenic basis [113]. However, activation of the mammalian target of rapamycin (mTOR) pathway observed in the tuberous sclerosis complex is not present in focal cortical dysplasia [8, 80]. The giant neurons and ballooned cells, which are histopathological features of tuberous sclerosis and focal cortical dysplasia, were absent both in the subependymal nodules and in the focal cortical dysplasia observed in the examined autistic cohort. These findings suggest that in spite of similarities, the pathomechanisms of developmental alterations are different in the examined autistic subjects than those in tuberous sclerosis heterotopias or focal cortical dysplasia. The development of the giant neuron- and balloon cell-free dysplasias observed in the autistic subjects might be related to differences in cause and/or mechanism. The detection of changes similar to focal cortical dysplasia in association with prenatal ischemia [65] or in shaken infant syndrome [74] may support these speculations.

Ectopias and dysplastic changes were reported in the brains of autistic subjects, by several groups [4, 62–64, 91]. Bailey et al. [4] detected olivary dysplasia in the brain of three of the five autistic subjects, and ectopic neurons related to the olivary complex in two cases. Moreover, in the brains of four autistic subjects, cortical dysgenesis was found. In the brains of the autistic subjects, a strikingly consistent finding was cingulate cortex disordered lamination [62–64, 100]. A recent study of the cingulate cortex of nine autistic subjects revealed a developmental malformation with irregular lamination in three cases, and an increased number of neurons within the subcortical white matter in two [100]. Simms et al. [100] suggest that the excessive number of neurons in the subcortical white matter reflects the lack of proper resolution of the transient zone in the developing brain of autistic subjects. Studies by Fatemi et al. [37, 38] link the migration and lamination defects to a striking reduction of reelin (by 40%) and Bcl-2 (by 34–51%) in the brains of autistic subjects. Our studies along with others' suggest that in the majority of autistic subjects, heterotopias and dysplastic changes are the local sign of general developmental defects of migration with a broad spectrum of topographic, morphological, and functional outcomes.

In the examined brains of autistic subjects, signs of neuronal immaturity were a common finding. Failure of maturation of neuronal precursors caused by altered expression of cytoskeletal proteins and loss of neuronal polarity results in defects in migration to the destined layer and in incorrect vertical and horizontal orientation [93]. The immaturity of dysplastic neurons is reflected in the expression of a variety of proteins and mRNA that are not present in mature neurons an altered expression of developmentally regulated cytoskeletal elements [3, 23, 61, 76],

which are known to be crucial for dendrite arborization, spine formation, axon outgrowth and maintenance of cell size and shape. Reduced cell size, dendritic arborization and spine expression are characteristic of dysplastic neurons [6, 93]. Cortical dysplasias are the most epileptogenic lesions of the brain [107] and are observed in up to 25% of all epileptic surgeries [102]. More subtle cortical malformations or dysgenesis encountered in adults with epilepsy may lack the histological criteria for focal cortical dysplasia. They have been described as mild cortical dysplasia or microdysgenesis [77].

Microdysgenesis within the entorhinal cortex of the 23- and the 60-year-old autistic subjects in the examined cohort is unique because the selective deficit of neurons was limited almost exclusively to the stellate neurons in the second layer. It is possible that the observed dysgenesis is a result of defective migration of neurons to their intended destinations. The presence of a thicker molecular layer and the deeper location of islands in the entorhinal cortex of subjects with schizophrenia were previously interpreted as evidence that the stellate neurons do not reach their destinations during development, probably due to abnormal migration [36, 57]. Studies indicating the involvement of reelin and Bcl2 genes in the pathogenesis of schizophrenia [37, 47, 60] and the reduced expression of reelin and Bcl2 in people with autism suggest that these two genes play a role in abnormal brain development and contribute to the structural and functional anomalies seen in autism and schizophrenia [37].

The distortion of dentate gyrus development detected in two autistic subjects was reflected in granule cell migration into the molecular layer and formation of an additional granule cell layer. Distortion of the shape of the dentate granule cell layer with the formation of irregular circles and loops appears to be another piece of evidence suggesting abnormal neuronal migration and networking. Numerous factors up-regulate neurogenesis in the hippocampus [32], including seizures [70, 71], antidepressant drugs [59, 72] and lithium [18]. Several areas of dysplastic changes with significant deficits of pyramidal neurons were found in the CA1 sector in three autistic subjects, but thickening of the pyramidal layer and an increased packing of dysplastic neurons in the CA1 sector of the 56-year-old subject suggests a diversity of CA dysplasia patterns in autism. The lack of gliosis indicates that the observed pathology is a sign of microdysgenesis rather than an effect of hypoxic neuronal loss. A significant deficit of mature pyramidal neurons and the presence of small irregular or poorly differentiated oval neurons suggest the defect of neuronal maturation in autism.

We report a spectrum of focal developmental changes seen in the cerebellum of eight autistic subjects, including nodular (lobe X) [97] dysplasia in the cerebellum in five, vermal dysplasia in one, severe focal hypoplasia in one, and heterotopias in one other subject. The presence of heterotopias only in one control subject is evidence of a strong tendency for focal developmental changes of cerebellar microarchitecture that were present in 61% of the autistic subjects. Flocculonodular dysplasia affecting almost the entire lobe indicates that mechanisms leading to focal dysplasia, which were present in five (38%) of the autistic subjects, show extremely strong topographic predilection. The observed focal dysplasia was associated with profound local disorganization of granule cells, Purkinje cells and molecular layers limited to a small cerebellar compartment receiving major projections from the vestibular complex involved in the oculomotor and postural system. Similar cerebellar dysplastic changes classified as heterotaxias (clusters of poorly organized mixed cells) were identified in 14% of normal infants but in 83% of infants with trisomy of different chromosomes [92]. The presence within the dysplastic nodule of both GABAergic Purkinje cells produced from the cerebellar ventricular zone, and the glutamatergic granule neurons produced from the rhombic lip, and the preservation of the cytoarchitecture in the adjacent cerebellar folia suggest that the final steps of migration and networking are disturbed mainly or exclusively in the nodule of the majority of autistic subjects. The characteristic feature distinguishing lobule X from the other lobules is the abundance of the transcription factor Tbr2 positive unipolar brush cells (UBCs) [30, 34], which amplify inputs from vestibular ganglia and nuclei, by spreading and prolonging excitation within the internal granular layer [84]. Abnormal networking of Purkinje cells, granule neurons, and UBCs may contribute to altered cerebellar coordination of locomotion and motor learning and planning, as well as of higher cognitive processing [58]. Flocculonodular dysplasia appears to be another sign of the mosaic of local developmental defects, most likely predetermined by the spatial patterning of germinal zones in developing rhombic lip [110], and coexisting with more general developmental defects resulting in the accelerated growth of the brain in early childhood [89], minicolumn pathology [13, 14], reduced neuron volume [7, 108, 111], and desynchronized neuronal growth in many brain regions [111] observed in autism.

Identification of sub-groups with signs of hyperplasia, hypoplasia and normal-sized cerebellum [95] reflects the heterogeneity of the autistic population. Piven et al. [87] reported that cerebellar volume correlates with an increased total brain volume. In the majority of autistic subjects, reduced size of the cerebellar hemisphere is observed [42, 82], but this trend is not detectable in cohorts of high-functioning autistic individuals [56]. Regional hypoplasia affects the vermis in autistic individuals relatively often [20, 22, 52] and may be associated with the

deficits in attention-orienting [49, 104], stereotypic behavior and reduced exploration observed in autism [86]. In the examined autistic cohort, selective and severe hypoplasia of lobes 1–4 associated with hypoconvolution of a large portion of the dentate nucleus appears to correspond to clinically detected defects of movement coordination. These findings suggest that differences in the type, topography and severity of cerebellar developmental defects may contribute to different clinical manifestations.

In the 4–7-year-old autistic children examined in this study, the volume of the Purkinje cells was 38% smaller than that of the age-matched control group [111]. Moreover, it has been reported that Purkinje cells of the autistic subjects revealed a 40% decrease in the expression of glutamic acid decarboxylase 67 (GAD67) mRNA [114]. In autism, the basket cells provide an increased GABAergic feed-forward inhibition to Purkinje cells. The result could be disruption in the timing of Purkinje cell firings and altered inhibition of the cerebellar nuclei, which could directly affect cerebello-cortical output and contribute to the changes in motor behavior and cognition observed in autism [115]. These findings and the reduced volume (by 26%) of the neurons of the dentate nucleus seen in the 4–7-year-old autistic children [111] suggest that in autism, interactions between the Purkinje cells and dentate nucleus are modified on the structural, molecular and functional levels.

The (a) detected changes within the subependymal cell layer with subependymal nodular dysplasia, (b) subcortical and periventricular heterotopias and (c) neocortex, archicortex, dentate gyrus, cornu Ammonis and cerebellar dysplasia reflect focal modification of neurogenesis, migration and alterations of the cytoarchitecture of brain cortex, subcortical structures and cerebellum in autism. Detection of dysplastic changes only in one control brain and of the broad spectrum of focal developmental alterations in the brains of 92% of the autistic subjects indicates that focal changes are a reflection of global developmental abnormalities and that regional changes may have their own contribution to the clinical heterogeneity of autism.

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